

Cryosurgery of Intraepithelial Neoplasia of the Uterine Cervix

The results of a longitudinal
study and clinical aspects

By
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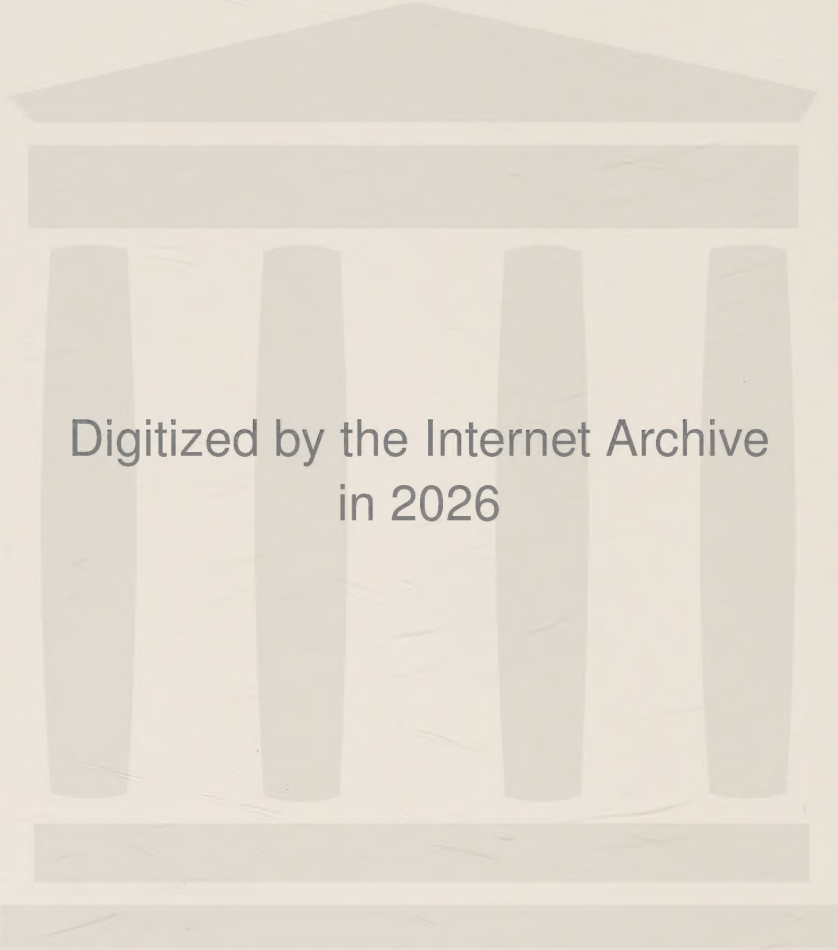
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ABBREVIATIONS AND DEFINITIONS. STATISTICAL METHODS.

Adiabatic process	A reversible thermodynamic process occurring without gain or loss of heat
CFR	Corrected failure rate
CIN 1	Cervical intraepithelial neoplasia grade 1 (mild dysplasia); undifferentiated neoplastic cells occupying up to one third of the thickness of the epithelial layer
CIN 2	Cervical intraepithelial neoplasia grade 2 (moderate dysplasia); undifferentiated neoplastic cells occupying up to two thirds of the epithelium
CIN 3	Cervical intraepithelial neoplasia grade 3 (severe dysplasia and carcinoma in situ); neoplastic changes as lack of maturation, pleomorphism, proliferation and loss of polarity of the cells occupying more than two thirds and extending through the entire thickness of the epithelium
ECC	Endocervical curettage
Eutectic point	The lowest possible temperature of solidification for any mixture of specified constituents
FR	Failure rate

Isentropic process	Reversible adiabatic process without change in entropy
IUD	Intrauterine device
LMP	Last menstrual period
NFR	No-follow-up rate
PFR	Preliminary failure rate
PID	Pelvic inflammatory disease
RFR	Real failure rate
Squamo-columnar junction	The line of demarcation separating original squamous epithelium from original columnar epithelium
Transformation zone	The area extending from the original squamous epithelium to the border between metaplastic epithelium and the original columnar epithelium
Unsatisfactory colposcopy	The squamo-columnar junction or the transformation zone not visualized

STATISTICAL METHODS. The following techniques were used: Chi-square test, Student's t-test, life-table calculation and discriminant analysis. All significance tests two-tailed.

INTRODUCTION

In Sweden, as in other Scandinavian countries, diagnostic and usually also therapeutic cold-knife conization is the most commonly used method for treating patients with cervical cytologic smears indicating severe dysplasia or carcinoma in situ (2, 12, 42, 50, 72, 89, 92, 98, 100, 157). In many other countries, conization is as a rule followed by total hysterectomy with or without a vaginal cuff. The reason for such a treatment regime is that residual neoplasia has frequently been found in hysterectomy specimen after previous conization (79, 142). Conization is not a harmless procedure. It requires the resources of a hospital, and its high rate of complications is well documented. Conization is generally not performed unless the cervical smear or cervical biopsy suggests severe dysplasia or carcinoma in situ. In cases of mild or moderate dysplasia, the patients are usually checked with repeated cervical smears at short intervals until indication for conization appears.

After population screening for precursors of cervical carcinoma was introduced in Sweden in the middle of the 1960's, the number of identified cases of precursors of cervical carcinoma has increased, especially in younger women (44, 53). The number of cases of carcinoma in situ was about 3,500 per year in the middle of the 1970's (118, 119). With the increased use of colposcopic examination of patients with abnormal cervical smears, a more conservative attitude toward the treatment of preinvasive carcinoma developed. Thus, for example, electrocoagulation diathermy, which is an out-patient procedure requiring general or regional anaesthesia, has been reported to yield good results (23, 75, 83, 124, 125, 135). Cold coagulation, i.e. a temperature of +70°C to +90°C has also been used to a minor extent (143, 152). The most recent technique is the use of laser therapy in cervical dysplasia and carcinoma in situ. As yet, experience with its effectiveness is limited, and it requires further evaluation (8, 9, 21, 38, 101, 139, 151). In selected cases, local excision alone can be an alternative to other conservative treatments (3).

Decreasing economic resources and rapidly escalating costs of hospitalization make development and evaluation of simple, effective and inexpensive out-patient therapeutic methods urgent. One method that appears to fulfil these requirements is the cryosurgical method. The equipment required for this method is easily handled (28, 158).

As cervical neoplasia is a disease considered to usually have a long natural history, the trend during recent years has been to choose initially a conservative method of treatment and to restrict the use of a more radical treatment to those cases where the conservative method fails. An important goal is to find a highly effective conservative method of treatment which could be instituted early and individually adjusted, and which was associated with minimal risk of physical and psychologic trauma to the patient. It should also be emphasized that whatever method is used, a careful, appropriate and long-term follow-up is mandatory (15, 19, 29, 31, 91, 98).

A REVIEW OF THE LITERATURE REGARDING RESULTS OF CRYOSURGICAL TREATMENT OF SEVERE DYSPLASIA AND CARCINOMA IN SITU OF THE UTERINE CERVIX

General remarks

Only investigations which primarily report experience with cryosurgical treatment of severe dysplasia and carcinoma in situ are reviewed. Minor attention is paid to cryosurgery in less advanced disorders of the uterine cervix, as there is generally a high rate of success in treating these cases (7, 31, 71, 76, 80, 85, 87, 128, 130, 141, 163, 165, 169). Throughout this review results are presented in terms of failure rate (FR) after one cryosurgical treatment and corrected failure rate (CFR) after more than one cryosurgical treatment session. Multiple freezing indicates more than one freezing but at different sites. As a variety of criteria are used in the literature for definition of both failure and cure rate, the results are often difficult to evaluate and compare across studies. Two common problems in assessing the reported efficacy of this treatment are too short periods of follow-up and an extensive number of drop-outs. Yet another problem is that the pretreatment evaluation by biopsy or shallow conization per se may be therapeutic, thus giving a false impression of the effectiveness of cryosurgery.

The first report dealing with cryosurgical treatment by means of modern equipment in a patient with carcinoma in situ of the uterine cervix was published by Cahan in 1964 (20). At panhysterectomy eight weeks after the treatment, no persisting disease could be observed. The first Scandinavian report of cryosurgical treatment of severe dysplasia and carcinoma in situ was presented by Sténson et al. in a lecture at the Nordisk Cancerunions symposium 1972 in Oslo (155). Since that time, the literature on this topic has expanded considerably. For the sake of clarity in this review, literature reports fulfilling similar criteria of pretreatment evaluation and/or cryosurgical technique are assigned to different subgroups and summarized in Tables 1-8.

Pretreatment evaluation by cervical biopsy

Three of the investigations presented in Table 1 are from the early 1970's when colposcopic assessment of the lesions was less common. It is noteworthy that in all of these studies the single-freeze and the double-freeze technique are used inconsistently.

Pretreatment evaluation with colposcopic assessment of the lesions and colposcopically directed biopsy

Two of the reports (Underwood et al. 1976, Boelter et al. 1978) presented in Table 2 with a failure rate below 10 per cent included treatment of slight to moderate dysplasia. Using a single-freeze technique resulted in a failure rate of 24-66 per cent in a population characterized by a short follow-up and a high drop-out rate (Leiman et al. 1980, Block et al. 1980). In two reports with no observed failures, the number of patients treated was small and the follow-up short (Kohan et al. 1977, Keefer et al. 1979).

Miscellaneous pretreatment evaluation

In one of the investigations (Saidi et al. 1977) shown in Table 3 which studied nine patients with moderate dysplasia and four with severe dysplasia, 46 per cent progressed to a more advanced degree of neoplasia subsequent to cryosurgery. By postponing the second freezing treatment for two months, the heavy vaginal discharge was reduced to only 30 per cent of cases as compared with 70 per cent of cases with two freezings at the same session. In addition, the patients were reevaluated colposcopically before the second application (Selim et al. 1980).

Cryosurgery in patients fulfilling special criteria

In all of the investigations cited in Table 4 there was a careful, colposcopically directed biopsy and ECC, procedures that might frequently have removed the lesions. It is obvious from these studies that treatment of patients with positive ECC gives a higher failure rate than does treatment of patients with negative ECC (Kaufman et al. 1978, Hatch et al. 1981)

Pretreatment evaluation by shallow conization

The authors cited in Table 5 conclude that the diagnostic procedure might have removed the abnormal tissue in about 75 per cent of cases. The low failure rate is probably due to the combined effect of treatment by conization and cryosurgery.

Conization or hysterectomy subsequent to cryosurgery

In all of the investigations shown in Table 6, the patients were evaluated by cervical biopsy before treatment. Single-freeze technique seemed to yield a high failure rate which nevertheless was reduced to one fourth with the double-freeze technique (Creasman et al. 1973). Operation as early as 6-8 weeks after cryotreatment revealed persistent disease at the same high frequency as did hysterectomy after conization.

Table 1. Pretreatment evaluation by cervical biopsy

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Kaufman et al. 1971 (85)	29	14	Freon 3 or 5 min or 3 min x 2	Colposcopically directed biopsy. Failures diagnosed by cytology or histology. All failures treated only 3 min. Length of follow-up 2 to 24 months.
Tredway et al. 1972 (165)	49	30.6	Freon 2-3 min or 2-3 min x 2 or ice ball 2-3 mm beyond the lesion	Colposcopically directed biopsy and endocervical curettage (ECC). Only patients with negative ECC treated. Two unsuccessful treatments required for defining failure (diagnosed by cytology and histol- ogy). Length of follow-up average 6 months.
Kaufman et al. 1973 (87)	49	24.5	Freon 3 or 5 min or 3 min x 2	Colposcopically directed biopsy and ECC. Only patients with negative ECC treated. Failures diagnosed by cytology or histology. Length of follow-up 2 to 48 months.
Henriksen 1979 (71)	29	17	-72°C 4 min. Ice ball 4 mm beyond the probe tip. A few patients treated with double- freeze technique.	Diagnostic curettage and/or biopsy. Failures diagnosed by biopsy. Length of follow-up 2 to 7 years.

Table 2. Pretreatment evaluation with colposcopic assessment of the lesions and colposcopically directed biopsy

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Creasman et al. 1975 (31)	179	22	-60°C 3 min x 2	Only patients with agreement among cytology, colposcopy and histology treated. Corrected failure rate (CFR) 13 %. Failures detected by cytology or biopsy. Length of follow-up minimum 6 months.
Underwood et al. 1976 (168)	22	6	Freon or nitrous oxide. Double-freeze technique. Ice ball 2 mm beyond the lesion. Multiple freezing.	ECC performed on each patient. 38 patients with mild-moderate dysplasia included in results. CFR 3 %. Failures detected by cytology. Length of follow-up less than 1 year in 50 %, more than 2 years in 17 %.
Kohan et al. 1977 (90)	26	0	Nitrous oxide 3 min x 2	Only young, nulliparous patients treated. Length of follow-up "admittedly short".
Popkin et al. 1978 (130)	75	6.7	Nitrous oxide 3 min x 2. Multiple freezing.	ECC if entire lesion not visualized. Failures detected by histology. Length of follow-up 9 months to 2.5 years.

(cont.)

Table 2. (cont.)

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Boelter et al. 1978 (17)	8	8	Nitrous oxide 3 min x 2	Patients with positive ECC not treated. 87 patients with slight-moderate dysplasia included in results. Failures detected by cytology or histology. Length of follow-up 3 to 18 months.
Crapanzano 1978 (30)	9	16	-60°C. Double-freeze technique. Ice ball 5-6 mm beyond the probe.	Results included 23 patients with slight-moderate dysplasia. Failures detected by cytology.
Keefer et al. 1979 (88)	8	0	Not recorded	Following treatment all patients had negative cytologic and colposcopic findings. Length of follow-up 6 months to 2 years.
Leiman et al. 1980 (103)	136	66.2	Nitrous oxide. Single-freeze technique. Ice ball 3 mm beyond the lesion.	ECC not performed. CFR 53 %. Failures detected by cytology. Length of follow-up 6 to 21 months (mean 7 months). 15.5 % failed to return after treatment.
Block et al. 1980 (16)	180	24	Carbon dioxide. Single-freeze technique 4-6 min.	34 % not checked after initial treatment. Length of follow-up one year in 29 % of cases.

Table 3. Miscellaneous pretreatment evaluation

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Stendahl et al. 1974 (153)	59	20.3	Liquid nitrogen 30 s x 2. Endo- and ectocervical application.	Iodine-directed biopsy and ECC. Patients with endocervical lesions treated. CFR 1.7 %. Failures detected by cytology. Length of follow- up 6 to 18 months.
Saidi et al. 1977 (140)	4	100	Freon or nitrous oxide 5 or 6 min. Double-freeze technique. Ice ball 3 mm from cryoprobe.	Colposcopically directed biopsy. ECC if no lesion visible. Failures diagnosed by cytology and histology. Length of follow-up average 6 months.
Lickrish et al. 1977 (107)	108	10.2	Nitrous oxide 3 min x 2	Colposcopically directed biopsy. ECC when necessary. Only patients with lesion entirely seen or negative ECC treated. CFR 6.5 %. Length of follow- up less than 1 year in about 60 %.
Walton et al. 1980 (169)	82	31.6	Nitrous oxide -60°C 5 min or -85°C 3 min x 2	Colposcopically directed biopsy. Pa- tients with positive ECC or lesions extending into the endocervical canal not treated. Abnormal cytology 3 months after treatment defined as a failure. Length of follow-up 2 to 49 months (mean 23.7 months). No follow-up in 7 %.
Charles et al. 1980 (24)	15	44.4	Freon 5 min. Ice ball 4 mm beyond the edge of the lesion.	Satisfactory colposcopy. Lesions in- volving more than 50 % of exocervix not treated. Failures diagnosed by cytology or biopsy. Length of follow- up 6 months to 3.5 years; 40 % drop- out. (cont.)

Table 3. (cont.)

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Kulseng-Hanssen et al. 1980 (99)	86	38.4	Freon or nitrous oxide 3 or 5 min or 3 min x 2	Colposcopically directed biopsy. ECC in most patients. FR with double-freeze technique 23 %, 7 % if patients with positive ECC were excluded. Failures detected by cytology. Length of follow-up 1 to 7 years.
Selim et al. 1980 (141)				
Study part I	41	9.8	Carbon dioxide 3 min x 2	Multiple biopsy and ECC. No colposcopy.
Study part II	37	5.4	3 min and 8 weeks later 3 min	Satisfactory colposcopy, biopsy and ECC. Failures diagnosed by cytology and/or histology. Length of follow-up 6 months to 7 years.
Kristensen et al. 1981 (96)	90	12	Nitrous oxide 3 min and 1-3 months later 3 min or 3 min x 2	Colposcopically directed biopsy. ECC in a few cases. CFR 8 %. Failures diagnosed by cytology. Length of follow-up 2 to 6 years.
Charles et al. 1981 (25)	39	18.2	Carbon dioxide or nitrous oxide 3 min x 2. Ice ball 4-5 mm from the edge of the lesion. Multiple freezing.	Satisfactory colposcopy, directed biopsy. Failure defined as cytologic or histologic CIN 4 months after treatment. Length of follow-up 6 to 72 months. No follow-up in 43.6 %.
Stuart et al. 1982 (156)	64	17.2	Single-freeze 5 or 7 min. 6 weeks later a second freeze in selected cases.	ECC in most cases. Colposcopically directed biopsy. CFR 12.5 %. Lost to follow-up at 1 year 31.8 %.

Table 4. Cryosurgery in patients fulfilling special criteria

Cryosurgery was offered only to patients with 1) completely visible lesions on the exocervix, 2) visible upper limits of the transformation zone, 3) agreement between cytology, histology and colposcopy.

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Kaufman et al. 1978 (86)	126	18.3	Freon or nitrous oxide 3 or 5 min or 3 min x 2	Patients with positive ECC treated. FR 20.8 % with positive ECC, 5.8 % with negative ECC. Failures defined as persistent atypical cytology or persistent or recurrent atypical histology. Length of follow-up 2 months to 8 years.
Ostergard 1980 (128)	46	19.6	Carbon dioxide or nitrous oxide 3 min x 2 or ice ball several mm outside the lesion	Persistence defined as CIN diagnosed by cytology within 14 months after therapy and recurrence as CIN diagnosed more than 22 months after therapy. Length of follow-up one year in 44 % of cases.
Hatch et al. 1981 (69)	246	23.8	Freon or carbon dioxide 3 min or 3 min x 2 or ice ball 3 mm from the edge of the probe.	Patients with positive ECC treated. FR 62.5 % with positive ECC, 7 % with negative ECC. Persistence defined as histologic proof of CIN detected in either of the first two follow-up visits, recurrence as CIN appearing after two negative smears. Length of follow-up 3 to 95 months (median time 12 months). No follow-up in 27 %.

(cont.)

Table 4. (cont.)

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Javaheri et al. 1981 (80)	60	16	Carbon dioxide or nitrous oxide. Double-freeze technique. Ice ball 4-5 mm from the edge of the lesion.	Only patients with focal lesions and negative ECC treated. Persistence defined as CIN within 12 months, recurrence as CIN after the first year. Length of follow-up 1 to 5 years. No follow-up in 17 %.
Wright et al. 1981 (173)	44	25	Double-freeze technique 8 min	Failures diagnosed by cytology, confirmed by histology. Length of follow-up 12 to 42 months.
Benedet et al. 1981 (7)	365	8.5	Nitrous oxide. Single-freeze technique 3 min. Ice ball 3-4 mm beyond the lesion.	Successful treatment defined as normal cytologic and colposcopic findings at 1 year after treatment. All patients followed-up a minimum of one year except 4.9 % lost to follow-up.

Table 5. Pretreatment evaluation by shallow conization

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Crisp et al. 1967 (34)	9	5	Liquid nitrogen -120°C to -160°C 3 min x 2	Hysterectomy or radical operation 1 to 7 weeks post freezing. Treatment of 11 patients with Stage I carcinoma of the cervix included in FR. Longest follow-up 22 months.
Crisp et al. 1970 (35)	52	10.5	-60°C to -160°C 3 min x 2	Treatment of 53 patients with lower degree of dysplasia included in FR. Length of follow-up 35 months.
Crisp 1972 (33)	96	6.5	-160°C to -180°C 3 min or -60°C to -80°C 3 min x 2	Treatment of 66 patients with lower degree of dysplasia included in FR. CFR 0 %. Longest follow-up 65 months.

Table 6. Conization or hysterectomy subsequent to cryosurgery

Author	No. of patients	Failure rate (%)	Freeze technique	Remarks
Creasman et al. 1973 (32)	75	30	-60°C 3 min or 3 min x 2	FR 48.5 % at single-freeze technique, 18.7 % at double-freeze technique. FR 12.5 % in young, low-parity patients. Operations 6 weeks to 3 months after cryosurgery.
Nielsen et al. 1973 (122)	11	81.8	-80°C 3-4 min	Hysterectomy 4 to 10 weeks (average 6.5 weeks) after cryosurgery.
Bloch et al. 1980 (16)	111	68	Carbon dioxide 6 min. Single-freeze technique.	Improvement in 29 % of cases. Cone biopsy or hysterectomy 6 to 8 weeks after cryosurgery.

Cryosurgery in patients with abnormal cytologic findings after diagnostic biopsy

In those investigations shown in Table 7, the pretreatment evaluation was based on colposcopically directed biopsies in all but one study (Tronstad et al. 1980). In this study, multiple, extensive biopsies and ECC were performed under general anaesthesia without colposcopy. The above reports are similar in that persistent disease was diagnosed by abnormal smear subsequent to the diagnostic procedure. However, the results are not comparable across studies because of varying criteria for failure and varying follow-up time. At the XIX Nordic Congress of Obstetrics and Gynecology in Reykjavik in 1976, Stendahl (154) reported a primary failure rate of 22 per cent (CFR 9 %) subsequent to cryosurgical treatment of 137 patients with remaining positive smears (PAP. III or worse) after biopsy-confirmed diagnosis of dysplasia and carcinoma in situ. The latest follow-up data for the study reported by Einerth (1978) was given at the Ninth World Congress of Gynecology and Obstetrics in Tokyo in 1979 (41). A failure rate of 15.3 per cent (CFR 10.2 %) was found after cryosurgical treatment of 59 patients during a follow-up period of 12 to 52 months (mean 29 months).

Long-term studies

As can be concluded from Table 8, patients successfully treated by cryosurgery seemed to run no greater risk of developing a new disease than did untreated high-risk women in the general population.

Table 7. Cryosurgery in patients with abnormal cytologic findings after diagnostic biopsy

Author	No. of patients	Failure rate (%)	Freeze-technique	Remarks
Townsend et al. 1971 (163)	62	11.3	Liquid nitrogen 1.5-2 min or Freon or carbon dioxide 2-3 min x 2. Ice ball 3 mm beyond the lesion.	Lesions covered more than 40 % of exocervix in failures. All failures treated by liquid nitrogen. Failures defined as moderate or worse dysplasia diagnosed by cytology or histology. Length of follow-up 12 to 44 months.
Gray et al. 1975 (64)	10	20	Nitrous oxide 3 min x 2	Persistent dysplastic smears considered as failures. Length of follow-up 3 to 12 months.
Einert 1978 (40)	35	11.4	Carbon dioxide 3 min x 2	CFR 8.6 %. Failures diagnosed by cytology. Length of follow-up 7 to 37 months (mean 17 months).
Elmfors et al. 1978 (43)	91	18.7	Nitrous oxide 3 min x 2	CFR 9.9 %. Preliminary report.
Tronstad et al. 1980 (167)	78	34.6	Nitrous oxide 4-6 min. Single or double-freeze technique.	FR 30 % for exocervical lesions, 57 % for exo-and endocervical lesions. Failures discovered by cytology. Length of follow-up 7 to 36 months (average 18 months).

Table 8. Long-term studies

Author	Remarks
Richart et al. 1980 (136)	A multicenter study comprising 964 patients. After the pretreatment diagnosis of CIN 3, the cumulative rate of development of a new disease after successful cryosurgery was calculated. The cumulative rate of CIN 3 was 0.23 % at year 5, 0.24 at year 10 and 0.24 at year 14. The five-year follow-up comprised only 27 % and the ten-year follow-up 7.2 % of cases.
Hemmingsson et al. 1981 (70)	In 130 patients with pretreatment diagnosis of carcinoma in situ, the total failure rate was 18 % when followed-up at 5 to 8 years after treatment. All failures were diagnosed within three years of treatment, the majority of them (86 %) within the first year. The failure rate was 12 % in patients under 30 years of age and 23 % in those over 30.

Concluding remarks on literature

In summary, the results reported in the literature are difficult to compare with each other due to different diagnostic procedures, nonstandardized freezing techniques and varying posttreatment evaluation. Short periods of follow-up and varying definitions of failure add to the difficulties in evaluating the clinical effect of cryosurgical treatment. Yet, a total evaluation of the literature suggests that certain factors are of particular importance for the effect of cryosurgery in this context:

- treatment with the double-freeze technique usually gives better results than does the single-freeze technique (32, 85, 87, 99);
- repeated freezing sessions seem to increase the success rate (CFR) (31, 40, 41, 43, 96, 103, 107, 153, 154, 156, 168);
- colposcopic assessment of the lesions and directed biopsy give better results than does evaluation without colposcopy (141);
- treatment of exocervical lesions seems to give a higher success rate than does treatment of endocervical lesions (69, 86, 96, 99, 167);
- increased size of the lesions may be associated with a higher failure rate (86, 163);
- better results are achieved in younger women than in older ones (32, 70, 96, 103, 167).

In some studies the effect of cryotreatment is inconclusive because:

- conization or extensive diagnostic procedures prior to treatment may remove all lesions and therefore hinder proper evaluation of the effect of cryosurgery per se unless a repeat smear is taken (33, 34, 35);
- conization or hysterectomy subsequent to treatment gives no information about the clinical effect of treatment (16, 32, 122);
- a high percentage of treated patients is not included in follow-up examinations (16, 24, 103).

In order to evaluate the clinical effect of cryosurgery as the method of treatment, the following criteria should be fulfilled:

- histopathologically verified diagnosis of preinvasive carcinoma;
- cytologically and/or colposcopically verified persistence of disease prior to treatment;
- a standardized freezing technique used throughout the total investigation;
- a follow-up with cytology and histopathology;
- a large number of patients and a long, standardized follow-up period;
- uniform and clearly defined criteria for failure rate.

PURPOSE OF THE PRESENT INVESTIGATION

The general purpose of the study was, first, to evaluate the effects of cryosurgical treatment of patients with abnormal cytologic findings remaining after biopsy diagnosis of preinvasive cervical carcinoma and, second, to study the clinical effects of reducing the time interval between the initial abnormal smear and treatment by performing further diagnostic measurement after the first abnormal smear and in most cases cryosurgery at the same time as the biopsy.

The present study encompasses two periods of time, each covering three years. Period I includes the years 1973 through 1975, and Period II covers the years 1976 through 1978. The purpose of the Period I study was to evaluate the long-term effects of cryosurgical treatment of preinvasive carcinoma of the uterine cervix in women under 40 years of age. The study was conducted with the following conditions:

1. Cytologically severe dysplasia or carcinoma in situ.
2. Subsequent histopathological diagnosis of severe dysplasia or carcinoma in situ.
3. Remaining abnormal cytologic findings after the diagnostic biopsy and prior to the treatment.
4. A well-defined, standardized treatment procedure employed uniformly during the entire period of investigation.
5. Long-term follow-up by both cytology and histopathology.

In addition, both side-effects and complications, if any, as well as the possible influence on subsequent reproduction were studied.

The experiences obtained during study Period I suggested that a reduction of the time interval between the initial abnormal smear and the cryosurgical treatment might be made possible by performing diagnostic evaluation after the first abnormal smear. In most cases the interval was further shortened by performing cryosurgery at the same time as the biopsy. The purpose of the Period II study was to evaluate this simplified treatment in clinical practice.

In the present study, the term cervical intraepithelial neoplasia (CIN), as proposed by Richart 1969 (134), was consistently used to define and classify dysplasias and carcinoma in situ. CIN 1 corresponds to mild dysplasia, CIN 2 to moderate dysplasia, and CIN 3 to severe dysplasia and carcinoma in situ. This terminology is currently used in the international literature (69, 80, 96, 120, 127, 128, 133, 136, 147, 167, 169, 173). For further and more precise definition of the CIN classification, see p. 11.

CRYOBIOLOGY AND CRYOTECHNIQUE

A. Cryobiological aspects of cryosurgery

The mechanism of cryosurgical destruction is not yet fully understood. There is probably a combination of different cryobiological effects. Opinion differs about the principal factors contributing to the cellular death. However, there is general agreement about some of the mechanisms leading to cell destruction. The following is a brief discussion of some cryobiological factors contributing to the cryosurgical tissue destruction.

1. Physicochemical cryodestruction. When low temperatures are applied to living tissue, certain physical and chemical changes occur within the affected area:

Down to -5°C a cellular biologic system remains in a liquid state despite the cytoplasmic freezing point of -2.2°C . This is due to supercooling and a decrease of the freezing point by the various solutes.

Between -5°C and -15°C , ice crystals develop in the extracellular space followed by progressive hypertonicity, and the intracellular water remains supercooled and unfrozen (47). At this stage the cooling velocity plays an important role:

Slow cooling ($1^{\circ}\text{C}-5^{\circ}\text{C}/\text{min}$) results in the transfer of intracellular water to the extracellular space, and an intracellular toxic concentration builds up eliminating any intracellular freezing.

Rapid cooling ($> 100^{\circ}\text{C}/\text{min}$) results in intracellular crystals, as there is insufficient time for intracellular water to pass through the cell membrane and thus freeze in the extracellular space. Cells containing crystals may be considered as seriously damaged or dead (48, 60).

Below -15°C an isothermic transitional state occurs. Further increase of electrolytic concentration results in a decrease of the freezing point of the remaining unfrozen liquids. At a temperature below the eutectic point of the solutions, a solid state or complete crystallization is reached (-21°C for NaCl) (47, 111, 113). Opinion differs regarding whether the thermic and osmotic shock or the crystallization is the predominant destructive factor (48, 112). However, the crystallization causes the denaturation of lipid protein complexes along with membranous and intracellular structures. The cooling velocity determines the extent of cellular lesions occurring at subsequent thawing. After slow cooling, rapid thawing ($> 100^{\circ}\text{C}/\text{min}$) exposes the cell to a high

electrolytic concentration and increased temperature, inducing a migratory intra- and extracellular recrystallization by growth or aggregation of small crystals; however, the mechanism of recrystallization remains unclear (5). After rapid cooling, rapid thawing protects the cell from attaining a high and toxic electrolytic concentration and thus from recrystallization; slow thawing (1°C-10°C/min) exposes the cell to a high electrolytic concentration which could induce a migratory recrystallization phenomenon. The sequence of rapid cooling and slow thawing is generally maximally deleterious for the tissue (47, 49, 110, 111, 113, 162).

2. Vascular cryodestruction. In addition to the above factors, a cryoinduced thrombosis followed by ischaemic infarction is thought to contribute to cellular death (105). The cryothrombosis seems to result from different factors:

- constricted arterioles and venules due to hypothermia,
- modified vascular endothelium,
- increased permeability of the vascular walls with increased blood viscosity and decreased blood flow (microthrombi);
- increased activation of the contact factors resulting in the formation of platelet plugs (microemboli).

The appearance of microthrombi and microemboli in the vascular lumen reduces the circulation and leads to complete destruction of the tissue nutreated by the vessels. This hypothesis of vascular cryodestruction is supported by the fact that transplanted cells taken from a cryolesion immediately after thawing may be successfully retransplanted, whereas cells removed after 48 hours cannot be retransplanted (58, 105).

3. Immunologic cryodestruction. Explanations of the immunologic effects of cryodestruction are still in the hypothetical stage. It has been suggested that cryosurgery could increase the control of tumors by liberation of antigens but yet also support the growth of tumors by blocking antibodies (1, 46, 47, 49, 144). However, study of the clinical effects of cryoimmunization was not included in the present investigation and will not be further discussed.

B. Clinical signs of cryosurgery and related histopathology

The following facts regarding clinical signs of cryosurgery and related histopathology, which are reported in the literature, were confirmed in the present investigation.

During freezing a sharply demarcated ice ball is formed. When the probe is removed after thawing, the tissue appears ischaemic. When cellular destruction starts a few hours after treatment, histamine is released, the tissue becomes hyperemic and oedematous, and a heavy, watery vaginal discharge develops (34). During the first day after treatment, the exocervix is covered by a gelatinous membrane; the following day the membrane becomes more opaque, raised and sharply demarcated from the unfrozen tissue (84). Biopsy one day after treatment reveals extensive fibrinoid necrosis, oedema and infiltration of polymorphonuclear leukocytes. The vessel walls are thickened, and thrombi are noted within the vessels. A general weakness is reported to occur a few days after treatment and is considered to be due to loss of potassium secondary to cell destruction (26). The necrosis appears to reach its peak at about seven days after treatment. Fibrinoid degeneration of the vessel walls is observed, and no endothelial lining or surface epithelium is noted. After two weeks the gross appearance of the exocervix is that of dry gangrene with a black, wrinkled, dehydrated coagulum over the area of cryonecrosis. The frozen tissue starts to demarcate with a sharp histologic line between the necrotic and normal tissue. Beneath the fibrin membrane re-epithelialization begins with a thin layer of squamous epithelium covering the surface. Numerous capillaries and fibroblastic proliferation as well as infiltration of polymorphonuclear leukocytes are noted. The vaginal discharge gradually decreases during the following weeks. After three weeks the necrotic area reabsorbs, leaving a fresh, granulating surface. Four weeks following freezing the exocervix is covered by immature squamous epithelium, which is later replaced by normal stratified squamous epithelium (84). In most cases the cervix is healed by eight weeks after treatment. Histopathological examination of biopsy specimen taken years after cryosurgery may reveal hyalinized vascular channel or area of condensed stroma as the only residuals.

C. Equipment and applications of cryosurgery

Cryosurgery is defined as therapeutic application of cryogenic temperature for local destruction of living tissue. Physicists define the region of cryogenic temperature as extending downward from -150°C , but in medicine the term encompasses a range of temperatures above this, including the freezing point of cells and tissue (i.e. about -2°C) (57, 114, 162). The cryogenic temperature can be achieved in different ways, and there are two chief methods employed in cryosurgery:

1. Change of phase of cryogen. Liquid nitrogen is usually used as the cryogen, with a capability of freezing to -196°C . A change in phase from the liquid to gaseous state accounts for the cooling effect. The liquid nitrogen equipment has a great freezing capacity and was used in the initial period of cryosurgery (20, 33, 34, 35, 70, 153, 154). However, because of the size and expense of the equipment and the unstable nature of liquid nitrogen, this category of instruments has been replaced by a simpler technique.

2. Adiabatic, isentropic expansion of compressed gas (Joule-Thomson effect). Freon, carbon dioxide and nitrous oxide are the most commonly used cryogens with a freezing capability of -40°C , -78.5°C and -89°C , respectively. Instruments in this category utilize the cooling effect achieved when a compressed gas is allowed to expand through a small orifice (57). The equipment is easy to operate, and the cooling capacity is adequate for a majority of the procedures employed in gynaecology. Instruments of this kind have thus been used in most investigations during the last decade.

The extent of the cryosurgical destruction depends on a number of factors:

a. Temperature of the probe. The lower the temperature, the larger the size of the tissue destruction (28, 58, 168).

b. Size of the probe tip. The greater the diameter of the cryoprobe, the larger the ice ball (28, 58, 168).

c. Duration of freeze. The longer the freeze, the larger the tissue destruction (until freezing reaches a steady state) (58).

d. Number of applications. Repetitive freezings increase the volume of frozen tissue until a maximum is obtained, this probably due to increased thermal conductivity of the already frozen tissue (52, 59).

e. Temperature and density of tissue. Body temperature, vascularity and physical state of the treated tissue affect the size of the cryolesion (45, 58, 59, 74, 168).

The lethal effect of freezing is assumed to be reached at a temperature below -20°C (114). Thermal gradients of $10^{\circ}\text{C}/\text{mm}$ are usually obtained in cryosurgery. Thus, when using a probe temperature of -70°C , the ice ball must extend at least 2 mm beyond the lesions to ensure complete destruction of the lesions (111). When nitrous oxide is used as a refrigerant and a double-freeze technique is applied, destruction of tissue is obtained to a depth of 5-6 mm (17, 33).

In an extensive study by Anderson (1980), it could be shown that in 99.7 per cent of patients with CIN 3 the crypt involvement never exceeded 3.8 mm, and the distance between surface epithelium and the most deeply involved crypt did not exceed 5.2 mm (4). Thus, a destruction of the tissue to a depth of 5 mm would eradicate the lesions in almost all cases.

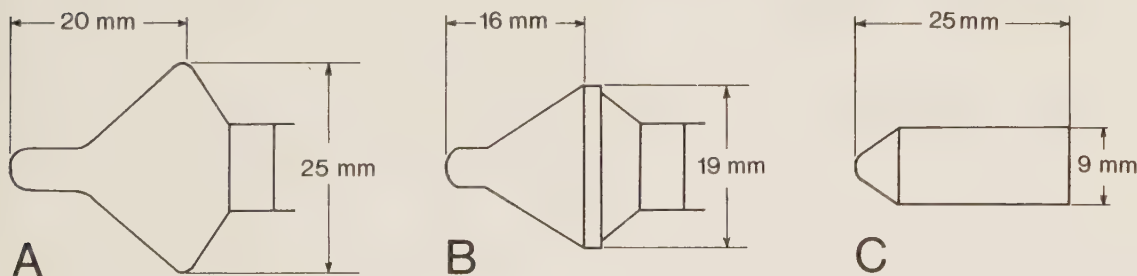
Techniques for monitoring the extent of the cryolesions include the use of hypodermic thermocouples, cryometer in the probe tip, visual assessment of the ice ball, palpation of the ice ball, or measurement of the time of application of the probe. Impedance measurements with hypodermic electrodes have recently been introduced to assess the extent of the ice ball (104). However, the use of thermocouples and electrodes is time-consuming and painful for the patient and thus not suitable for routine use. In the present investigation, as in most other studies, the extent of the cryolesion was monitored by measurement of the probe application time and by visual assessment. A double-freeze technique was chosen for the present investigation on the basis of theoretical considerations, and the results of a pilot study (Elmfors, unpublished data, 1972) of ten patients scheduled for operation of uterine prolapse. A cycle of three minutes freezing, three minutes thawing and three minutes freezing was thus employed. The two different instruments which were used are manufactured by Spembly Ltd. Andover, U.K. and are based on the Joule-Thomson expansion principle using nitrous oxide as refrigerant.

The TCC 10 cryosurgery system. The equipment consists of a control unit, gas supply, an ON/OFF footswitch and different probes. When the footswitch is depressed, the flow of gas under pressure is fed through the inner of two concentric tubes to a micro-orifice at its end, where it expands and causes a rapid drop in temperature. After expansion, the gas is returned along the outer of the two tubes to the control unit and exhausted. When the footswitch is released, the gas flow ceases and a low voltage electrical heater built into the probe tip is switched on to defrost the tip. A pear-formed probe with 25 mm diameter and 20 mm cervical extension was used in the present investigation (Fig 1. A). The average freezing rate is 5°C/s and its lowest tip temperature in air is -70°C. The probe and connecting hose are sterilized by autoclaving at a temperature of 120°C at 1.2 kg/cm² for 20 minutes. (Operating instructions, Spembly Limited).

The BMS 40 system. This equipment is non-electrical. During freezing it operates in a manner similar to that of the TCC 10 unit. During thawing a high-pressure defrosting technique is utilized. When the exhaust is blocked, the pressure of gas increases quickly and the cooling effect stops. Since the tip is very cold, gas condenses and the latent heat of condensation warms the tip, which can be removed from the frozen tissue in a few seconds. Two different probe tips were used: a pear-formed tip with 19 mm diameter and a 16 mm cervical extension (Fig. 1 B) or a cylindrical endocervical probe tip measuring 9 mm x 25 mm (Fig. 1 C). The probe tips, which are interchangeable, are sterilized by autoclaving at a temperature of 135°C at 2.4 kg/cm² for 10 minutes.

The nitrous oxide is stored in gas cylinders of 10 liters in the liquid phase at a pressure of 740 p.s.i. at an ambient temperature of 20°C. The instruments are designed to operate at 600-800 p.s.i (40-60 kg/cm²). When treating more than two patients in a row, full gas cylinders must be available in reserve because the extracted gas is replaced by evaporation of liquid leading to a successive drop in temperature of the cylinder, and a cold cylinder will not provide sufficient pressure for effective freezing.

Fig. 1. Configuration of probe tips used in the present investigation:
(A) 25/C/20 operates in conjunction with the TCC 10 system; (B) 19/C/16 and
(C) 9/C/25 operate in conjunction with the BMS 40 system.



Ventilation must be adequate in the treatment room, as exhausted gas may be hazardous to personnel working there. Exposure to high doses of nitrous oxide may cause impairment in cognitive, psychomotoric and coordinative performance. Recommended environmental concentration of the gas should not exceed 25 ppm during the period of nitrous oxide administration (18, 63). Carbon dioxide, an inert gas, can also be used in cryosurgical units but the freezing power is about 20 per cent below that obtained with the nitrous oxide.

PRESENT INVESTIGATION

I. PERIOD I STUDY

A. Design of the study

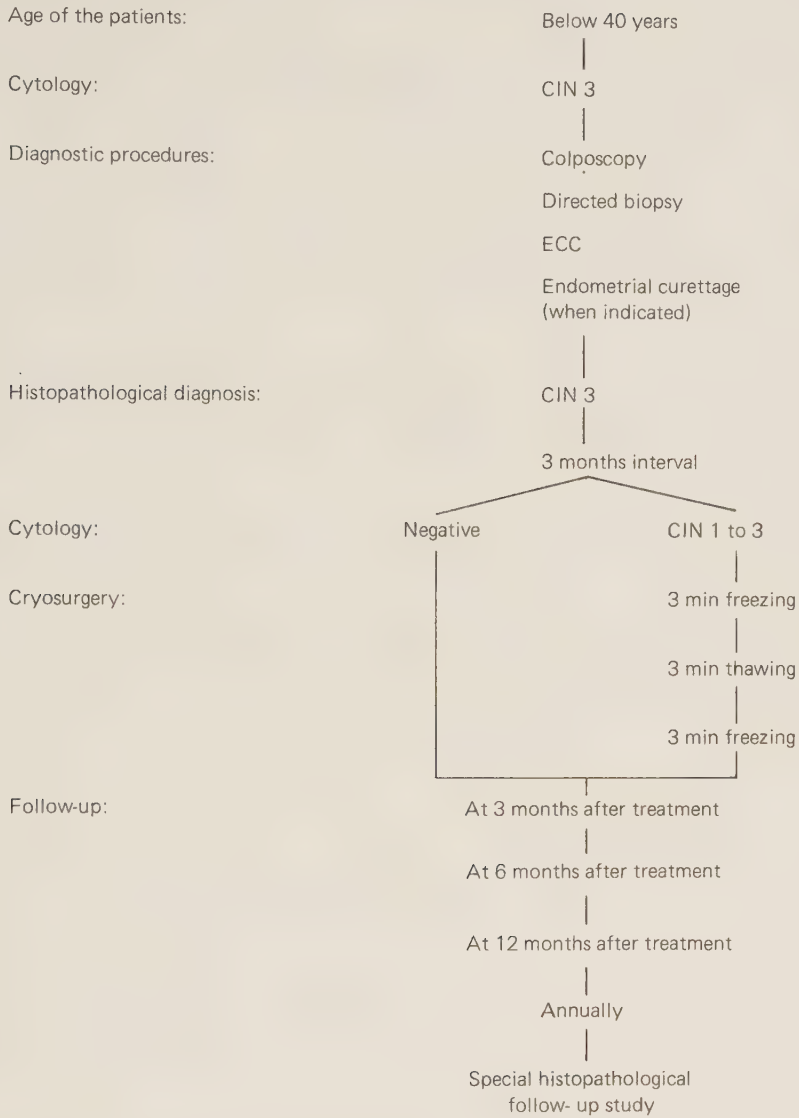
1. Management protocol

The purpose of the study was to evaluate the effects of cryosurgical treatment of patients below 40 years of age with a histopathological diagnosis of CIN 3. Thus, patients of all ages with invasive cervical carcinoma and patients with a histopathological diagnosis of CIN 3 who were 40 years or older were excluded from the investigation. Patients with a cytologic smear indicating CIN 3 were referred to diagnostic evaluation. The diagnostic procedures included colposcopic assessment, directed biopsy, ECC and, when indicated because of abnormal bleeding, endometrial curettage. Only patients with a histopathological diagnosis of CIN 3 were candidates for cryosurgery. After the diagnostic procedures were performed, repeated cytologic smears were taken. The patients were divided into two groups on the basis of post-biopsy cytologic findings:

1. Patients with persistently negative cytologic smears were not to be given further treatment.
2. Patients with persistent or recurrent disease were to be submitted to cryosurgical treatment.

The cryosurgical treatment was performed with nitrous oxide as refrigerant, using a double-freeze technique with two periods of three minutes' freezing, separated by three minutes' thawing interval. Follow-up of both groups included colposcopy, repeated cytologic smears and a special histopathological study comprising multiple biopsies and ECC. Patients with persistent or recurrent disease after the initial cryosurgical treatment were managed individually. The design of the Period I study is outlined in Fig 2.

Fig. 2. Design of the Period I study (1973-1975)



2. Definition of failure and failure rate

In the present study the definition of treatment failure was a cytologic and/or histopathological diagnosis of CIN 2 or 3 or repeated cytologic and/or histopathological findings of CIN 1 at any time during the follow-up period.

Failure rate was defined in the following manner:

Real Failure Rate (RFR) indicated the per cent of patients with failure in a period in which all the patients who were treated had been followed-up during an equally long time after treatment and no material attrition existed.

Preliminary Failure Rate (PFR) indicated the per cent of patients with failure among those who were followed-up in each period but where not all patients were followed-up. In each such period there was an increased number of patients who were not yet followed-up or who were lost to follow-up.

Corrected Failure Rate (CFR) indicated the per cent of patients with failure after more than one cryosurgical treatment. In the present study no more than two cryosurgical treatment sessions were performed in any individual patient.

No-Follow-up Rate (NFR) indicated the per cent of patients who were not yet followed-up or who were lost to follow-up in each period after treatment.

In addition, the cumulative proportion of patients with signs of persistent or recurrent disease after the diagnostic biopsy and after cryosurgical treatment are analysed and reported according to traditional methods of life table calculations (129).

B. Population and general remarks

The Department of Obstetrics and Gynaecology at the Central Hospital in Kristianstad serves three urban districts and four rural districts. The total area is characterized by relatively dense population with 46 inhabitants per square kilometer, as compared with 20 for the whole country. At the beginning of the investigation the total number of inhabitants in the area was 185,742; the total number of women was 92,790; the number of women above 20 years of age was 67,790. The rate of increase in the population was very uniform during the 1970's, representing an annual increase of 0.3-0.6 per cent (Fig. 3).

The total number of cases of cervical neoplasia (CIN 3 and invasive carcinoma) within the area and the incidence per 100,000 women, as well as per 100,000 women above 20 years of age during the Period I study, are shown in Table 9.

Fig. 3. Population and rate of increase in the area during the 1970's

Number in 1000's

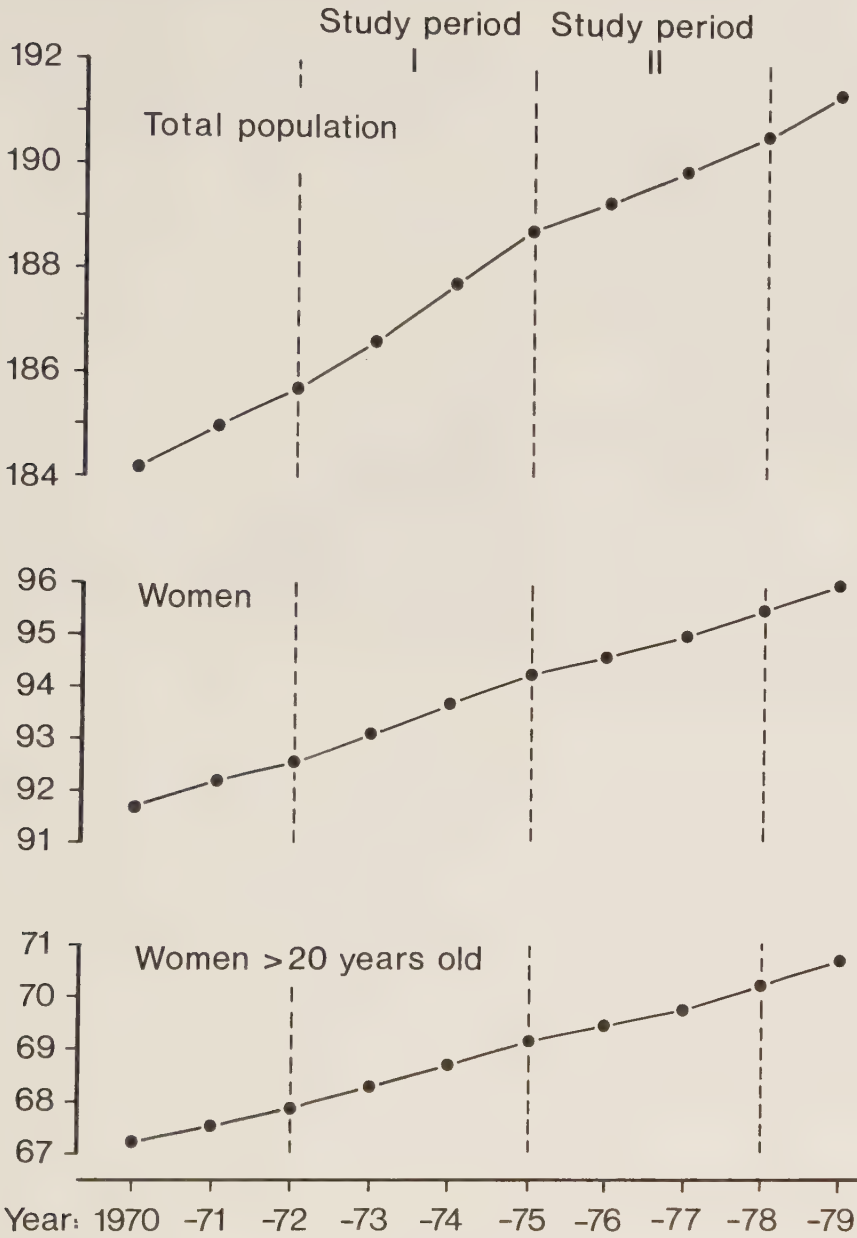


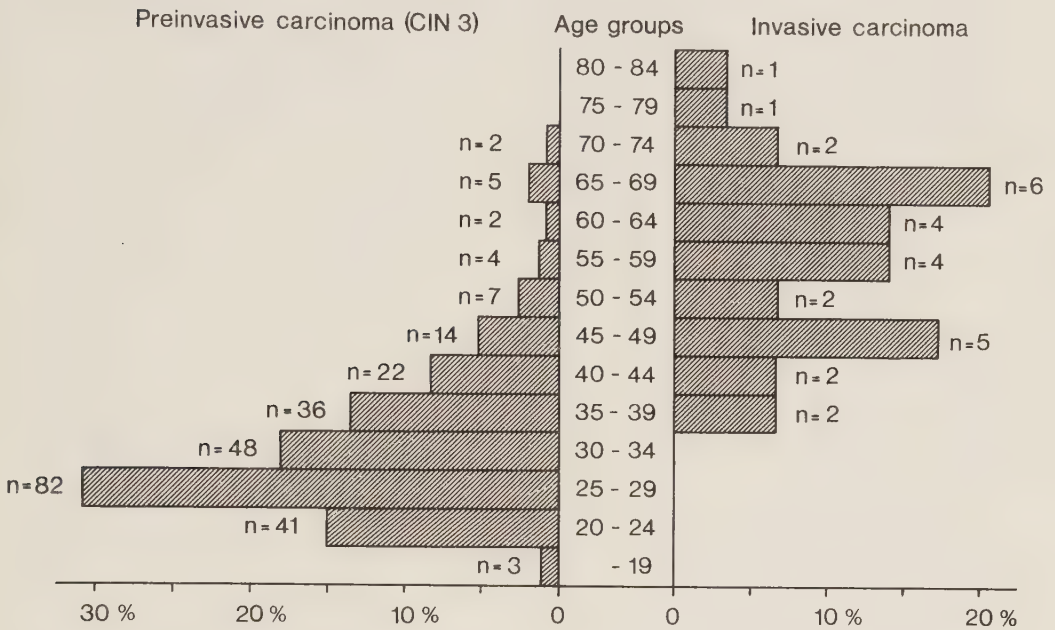
Table 9. Number of cases of cervical neoplasia (CIN 3 and invasive carcinoma) diagnosed at the Department of Obstetrics and Gynaecology, Kristianstad, 1973-1975 and incidence rate per 100,000 women and per 100,000 women above 20 years

Diagnosis	1973	1974	1975	Total
<u>Preinvasive carcinoma (CIN 3)</u>				
Number of patients	81	71	114	266
Rate per 100,000 women	87.1	75.8	121.0	94.6
Rate per 100,000 women >20 years	115.8	102.0	164.9	127.6
<u>Invasive carcinoma</u>				
Number of patients	6	13	10	29
Rate per 100,000 women	6.5	13.9	10.6	10.3
Rate per 100,000 women >20 years	9.0	19.3	14.5	14.2
Total no. of patients	87	84	124	295

As shown in Table 9, the mean rate of CIN 3 was 94.6 per 100,000 women and 127.6 per 100,000 women over 20 years. The corresponding figures for invasive carcinoma were 10.3 and 14.2, respectively, during the three-year period of the study. The age distribution of the patients at the time of histopathological diagnosis is shown in Fig. 4.

The 266 patients with a diagnosis of CIN 3 had a mean age of 33.7 years (range 18.2-74.6 years), and the 29 patients with invasive carcinoma had a mean age of 58.9 years (range 37.8-84.5 years). The mean age of the patients with CIN 3 agrees well with that in other reports from the same time period (72). In earlier investigations, however, the mean age was higher (11, 42, 89, 157). CIN 3 was predominantly found in the 25-29 year age group, as has also been found by others (39, 51, 72, 100). Of the 29 patients with invasive carcinoma, nine (31.0 %) were below 50 years of age and two (6.9 %) were below 40 years of age when the diagnosis was made.

Fig. 4. Percentages of patients with cervical neoplasia (CIN 3 and invasive carcinoma) by age and diagnosis (N=295) in the Period I study



C. Patients excluded from the present investigation

As mentioned above, patients with invasive cervical carcinoma and patients 40 years of age or older were excluded from the study.

1. Patients with diagnosis of invasive cervical carcinoma Stage I-IV

Even if the 29 patients with invasive carcinoma were excluded from the subsequent study, a few facts of clinical interest should be given about them.

In two patients (6.9 %) the carcinoma was found incidentally in the cervix removed at operation for prolapse of the uterus. The diagnosis was made after the discovery of abnormal routine cervical smears in 10 patients (34.5 %) examined for reasons unrelated to a cervical disorder and in the remaining 17 patients (58.6 %) when they sought medical advice for abnormal vaginal bleeding or heavy discharge in menopause. Two of the patients (6.9 %) had undergone a subtotal abdominal hysterectomy 21-22 years prior to the diagnosis of cervical carcinoma.

In 24 patients cervical smears were taken prior to or at the time of histopathological diagnosis. In 19 patients the smears were abnormal, while in the remaining five patients (20.8 %) the smears taken up to five months before diagnostic biopsy were negative (three of the five patients had adenocarcinoma).

Negative cytologic smears within five years prior to the detection of invasive carcinoma have been reported to have a frequency of 11-45 per cent (13, 39, 66, 78, 82, 115, 138). The presence of blood, infection, necrosis, discontinuous exfoliation of malignant cells, and rapidly growing tumors as well as errors in sampling and screening have been mentioned as possible causes of negative smears at invasive carcinoma (6, 54, 66, 67, 77, 78, 94, 115).

In 21 patients the diagnostic procedures were either cervical biopsy or cervical dilation and curettage, and in seven patients cold-knife conization or cervical amputation. One patient underwent total hysterectomy because of persistently abnormal cytology subsequent to conization.

The clinical stage of the disease and the histopathological diagnosis are shown in Table 10.

Table 10. Invasive cervical carcinoma in the Period I study. Clinical stage and histopathological diagnosis. The number of deaths during five years' observation given in brackets

Clinical stage	Total no.	Histopathological diagnosis	
		Squamous carcinoma	Adenocarcinoma
		No. patients	No. patients
I A	7	6	1
I B	8 (1)	5 (1)	3
II A	9 (5)	7 (3)	2 (2)
II B	2 (1)	2 (1)	
III - IV	3 (2)	1	2 (2)
Total no. of patients	29 (9)	21 (5)	8 (4)
Per cent		72.4	27.6

The percentage of patients with adenocarcinoma (27.6 %) is high, but others report increasing rates of adenocarcinoma; e. g. rates of 9-34 per cent are reported (15, 36, 39, 55, 65). All patients except three were treated primarily by radiation, which was followed in one case by total hysterectomy. Primary hysterectomy was considered adequate treatment for one patient, as was conization for two patients.

Two patients under 40 years of age were initially intended to be included in the Period I study but vessels highly suggestive of malignancy were detected at the colposcopic examination. The histopathological diagnosis of the specimens was adenocarcinoma in one of these patients and invasive squamous carcinoma in the other. In both patients, conization eradicated the disease.

Of the 29 patients with the diagnosis of invasive carcinoma, nine patients (31.0 %) have died at 5 to 34 months (mean 18 months) after the detection of the disease. The five-year survival rate was 50.0 per cent for patients with adenocarcinoma and 76.2 per cent for patients with squamous carcinoma (all stages).

2. Patients ≥ 40 years of age at the diagnosis of CIN 3.

Of the 266 patients with the histopathological diagnosis of CIN 3, 56 patients (21.1 %) were 40 years of age or more (Fig. 4). The reasons for excluding these patients from the present study were that there is an increased risk of invasive carcinoma in older women and that the transformation zone more frequently extends into the cervical canal and is therefore not colposcopically visible (126, 127, 140). As childbearing is generally completed at 40 years of age, more radical methods of treatment can be used after this age; conization was thus used in 42 patients (75,0 %), hysterectomy in six patients (10.7 %) and various methods of treatment in the remaining eight patients, as shown in Table 11. It should be mentioned that in most earlier reports of cryosurgical treatment of CIN 3, no upper limit was set for the age of the patients (32, 33, 35, 71, 86, 165).

Table 11. Therapeutic procedure or combined diagnostic/therapeutic procedures in women 40 years of age or older in the Period I study

Procedure	No. patients	Per cent
Conization after malignant cytologic smear	16	28.6
Conization after biopsy or cervical curettage	26	46.4
Total hysterectomy after biopsy or cervical curettage	4	7.1
Total hysterectomy after conization	2	3.6
Diagnosis incidentally found at treatment of prolapsus uteri	5	8.9
Biopsy followed by negative cytology	2	3.6
Radiation treatment	1	1.8
Total	56	100.0

D. Patients accepted as candidates for cryosurgery

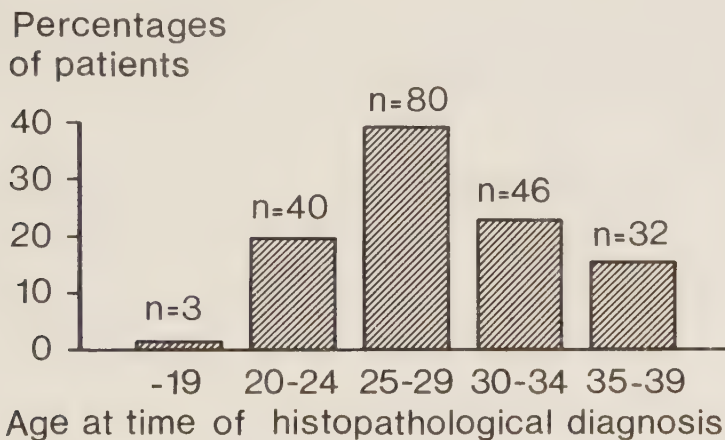
1. Present study material

The Period I study material was comprised of a total of 210 consecutive patients below 40 years of age at the histopathological diagnosis of CIN 3. However, nine of these patients had to be excluded from the study for different reasons (three patients moved out of the area, five underwent conization and one was hysterectomized because of fibromyoma). The remaining 201 patients thus comprised the final material in the present study.

a. Age distribution

The age distribution of the material at diagnosis is presented in Fig. 5.

Fig. 5. Age distribution of patients at time of histopathological diagnosis of CIN 3 in the Period I study (N=201)



The mean age was 29.2 years (range 18.2 - 39.9 years). In earlier studies mean age has varied between 25 and 42 years, although in most investigations mean age is about 30 years (30, 122, 141). The majority of the patients (61.2 %) were below 30 years of age at the initial histopathological diagnosis of CIN 3. This is in agreement with most other reports (7, 30, 32, 86, 130, 141, 168, 169, 170). Comparison of ages in the current versus other materials is not strictly appropriate, due to differences in criteria for material selection.

b. Obstetric background data prior to biopsy

The parity of the patients at diagnosis is listed in Table 12.

Table 12. Parity in 201 patients prior to the biopsy diagnosis of CIN 3 in the Period I study

Parity	No. patients	Per cent	Cumulative per cent
0 - para	35	17.4	17.4
1 - para	54	26.9	44.3
2 - para	67	33.3	77.6
≥3 - para	45	22.4	100.0
Total	201	100.0	

Forty-four per cent of the patients had no or only one child. Other studies report between 28-78 per cent with no or one child (30, 32, 168). In earlier reports on such material, extreme variability can be found for distribution of parity (30, 32, 35, 130, 170). The mean parity of 1.7 found in the present study was lower than that found in some earlier investigations (32, 103). The number of spontaneous and legal abortions is listed in Table 13. The mean number of pregnancies per patient was 2.1.

Table 13. Spontaneous and legal abortions prior to the biopsy diagnosis of CIN 3 in the Period I study

No. abortions	Spontaneous abortions	Legal abortions
	No. patients	No. patients
1	25	23
2	6	3
3	1	
Total no. of patients with at least one abortion	32	26

Age of the patients at the first pregnancy is listed in Table 14. Twenty-nine of the patients (14.4 %) had never been pregnant.

Table 14. Age at first pregnancy in 172 patients with the biopsy diagnosis of CIN 3 in the Period I study

Age at first pregnancy	No. patients	Per cent
<18	27	15.7
18 - 20	45	26.2
>20	100	58.1
Total	172	100.0

c. Contraceptive data prior to biopsy

As a number of patients in the study were on contraceptives with different methods which might possibly influence the cryosurgical treatment or the results, a few data will be given. Information about contraceptive methods used by patients is always uncertain. With this reservation, the contraceptive methods used by the patients in the present material prior to biopsy, as determined from hospital records and by asking the patients, are listed in Table 15.

Table 15. Reported contraceptive methods prior to biopsy in the Period I study

Method	No. patients	Per cent
Contraceptive pills	106	52.7
Barrier method	22	10.9
IUD	7	3.5
Varying methods	51	25.4
None	15	7.5
Total	201	100.0

The duration of using contraceptive pills or IUD before the diagnosis of CIN 3 is shown in Table 16.

Table 16. Reported total duration of contraceptive pills or IUD use prior to biopsy in the Period I study

Duration (in months)	Contraceptive method	
	Pills No. patients	IUD No. patients
1 - 11	21	12
12 - 23	20	9
24 - 47	36	6
≥ 48	70	2
Total	147*	29

* Includes some patients with varying methods (Table 15).

d. Genital infections prior to biopsy

A history of pelvic inflammatory disease (PID) was found for 28 patients (13.9 %). The majority of these patients (82.1 %) were treated as in-patients at the hospital. All were treated by broad-spectrum antibiotics for about two weeks. As routine use of laparoscopy in the diagnosis of PID was not introduced at the Department until 1976, PID was confirmed by laparoscopy in only one of the patients. Trichomonas infestation was diagnosed by wet smear test or by cytology in 21 patients (10.4 %). In 16 patients (8.0 %) a gonococcal infection was diagnosed by culture. A repeat gonococcal infection occurred in one patient.

2. Diagnostic approach

a) Cytologic history

The selection of the present material was based on abnormal cytologic smears found in women in the area served by the hospital. The reasons for taking those smears are listed in Table 17. Twenty-eight per cent of the patients were referred from the population screening because of abnormal cytologic findings. Ten per cent of the women in the study had not been invited to the screening program but had had a cytologic smear taken at their own request, and in 17 per cent cytologic smears were taken at the first check-up during pregnancy. All together about 80 per cent of the abnormal smears were detected at routine gynaecologic or obstetric check-ups not related to a disease. In addition to the 200 patients shown in Table 17, one patient was incidentally detected by histopathological examination of specimen from curettage following spontaneous abortion without a previous abnormal smear.

Table 17. Reasons for taking the smear at which cytologic abnormality was found in the Period I study (N=200)

Reasons for taking smear	No. patients	Per cent
Population screening	56	28.0
Routine gynaecologic check-up	20	10.0
Routine obstetric check-up	29	14.5
Request for legal abortion	5	2.5
Contraceptive advice	47	23.5
Complaints unrelated to a cervical disorder	30	15.0
Abnormal uterine bleeding	6	3.0
Other reasons	7	3.5
Total	200	100.0

Cytologic smears were taken prior to colposcopy and pelvic examination. Three specimens were collected in all cases: one specimen from the endocervix using a cotton-tipped applicator premoistened with saline; one specimen from the squamo-columnar junction; and one from the vaginal fornix, the latter two taken with an Ayre wooden spatula. The three specimens were applied on the same slide and fixed by SFIX^(R), a water soluble fixative (Polyethylene glycols and Ethylalcohol). Of the 200 patients - one patient caught by histopathology only - with abnormal cytologic smears entering the study for possible cryosurgery, the initial smear was negative in 90 patients (45.0 %) and abnormal in 110 patients (55.0 %). The classification of the initial smear in the 200 patients is given in Table 18.

Table 18. Classification of initial smear in the 200 patients who were candidates for cryosurgery in the Period I study

Cytology report	No. of cases	Per cent
Negative	90	45.0
CIN 1	34	17.0
CIN 2	48	24.0
CIN 3	28	14.0
Total	200	100.0

Women included in the population screening were referred for a new smear after four years, providing the first one was negative. However, every woman visiting an antenatal clinic or a gynaecologic out-patient clinic in the

region was subjected to a new smear whenever more than one year had elapsed since the last smear was taken. This explains why the 90 patients with negative initial smears were subsequently included in the present study. The change from negative to abnormal smears in those patients appeared after a varying number of examinations, as seen in Table 19.

Table 19. Number of patients with one to four negative smears prior to abnormal smear and average time from first negative to first abnormal smear in the Period I study

No. negative smears	No. patients	Per cent	Average time from first negative to first abnormal smear (in months)
1	53	58.9	32
2	23	25.6	53
3	11	12.2	62
4	3	3.3	83
Total	90	100.0	

At every new screening, about half as many patients with abnormal smears were detected as in the preceding screening. This finding is in agreement with an earlier report (145). The first abnormal smear which was found in these 90 patients indicated CIN 1 in 17 patients, CIN 2 in 35 patients and CIN 3 in 38 patients. In the total material, the first abnormal smear was classified in the manner shown in Table 20.

According to the design of the study, only patients with CIN 3 were to be subjected to further diagnostic evaluation and therapy. Patients with cytologic findings of CIN 1 and 2 underwent repeated smears. Because of the clinic's general policy during the early part of the present study period, even patients with CIN 3 sometimes were examined with repeated smears. Fig. 6 gives the number of abnormal smears taken before biopsy in the total material. Four and more cytologic smears were taken in about half of the patients with an initial smear indicating CIN 1 and 2. In contrast, 73 per cent of the patients with CIN 3 had less than four smears before biopsy. The mean number of abnormal smears taken before biopsy was 3.9, 3.8 and 2.8 when the initial smear indicated CIN 1, 2 and 3, respectively. It has been pointed out earlier

Table 20. Classification of the initial abnormal smear and average time from initial abnormal smear to biopsy diagnosis of CIN 3 in the Period I study

Cytology report	No. patients	Per cent	Average time from first abnormal smear to biopsy diagnosis of CIN 3 (in months)
CIN 1	51	25.5	29
CIN 2	83	41.5	23
CIN 3	66	33.0	16
Total	200	100.0	

that a correlation might exist between the number of abnormal smears in a patient and the risk for the lesions' progressing to carcinoma in situ (31). In the present study, the progression of CIN 1 and 2 to more advanced degrees was observed during the period of observation. Prior to biopsy, the distribution of the different degrees of neoplasia was that shown in Table 21.

Fig. 6. Total no. of abnormal smears per patient before biopsy in relation to cytologic classification of the initial abnormal smear in the Period I study

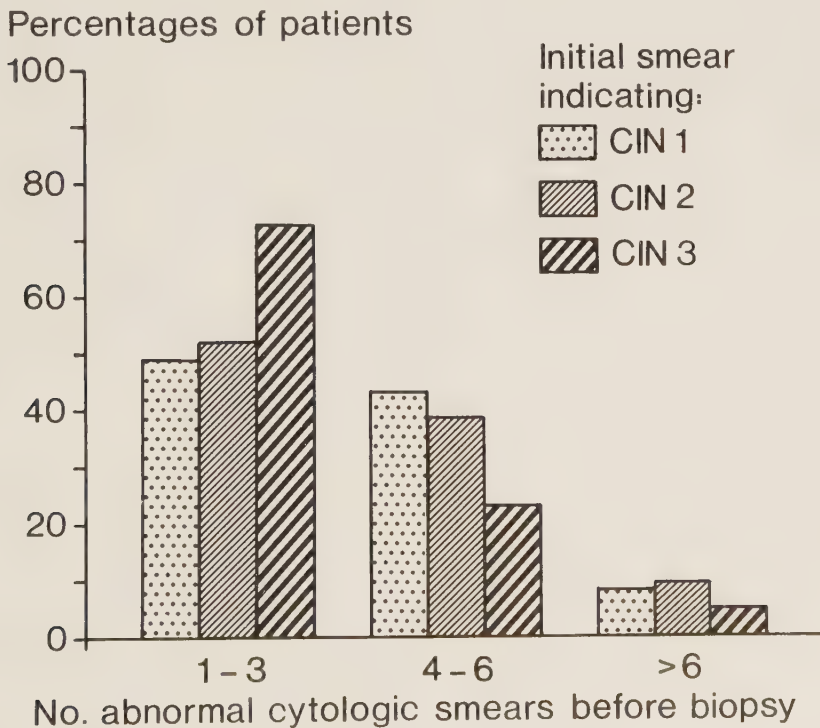


Table 21. Classification of most advanced cytologic abnormality prior to biopsy in the Period I study

Classification	No. patients	Per cent
CIN 1	7	3.5
CIN 2	48	24.0
CIN 3	145	72.5
Total	200	100.0

From Table 21 it would seem that the conditions of the basic design of the study were not quite followed, as even patients with CIN 1 and 2 were subjected to further diagnostic evaluation. However, it became obvious during the study that even patients with a lower degree of neoplasia were a risk group who could not be left without a histopathological diagnosis. Those patients with cytologic smears indicating CIN 1 and 2 and presented in Table 21 all showed CIN 3 at the biopsy diagnosis.

b) Diagnostic procedures

Patients selected for the present study according to the criteria mentioned above were referred to a specially established out-patient clinic for further evaluation and treatment. The procedures included biopsy (colposcopic examination technique was introduced during the early period of the study) and, as a rule, ECC. The latter was always performed on patients whose lesions extended into the endocervical canal, who had no visible lesions, or in whom the upper limits of the squamo-columnar junction or the transformation zone could not be fully visualized. An endometrial curettage was included when indicated because of abnormal bleeding. In such cases the procedures were performed under general anaesthesia in the operating theatre without the possibility of colposcopic examination. These patients were subsequently referred to the colposcopic clinic for further follow-up and treatment.

Technique of colposcopy. A self-retaining bivalve vaginal speculum (Handi-Spec. 28 mm x 90 mm LIC) was inserted into the vagina and the exocervix was exposed. The colposcope (Hinselman-Möller x 12) was focused on the exocervix. The transformation zone was inspected before and after a three per cent acetic acid solution was applied. Green filter was used for examination of the vascular pattern. The colposcopic examination was classified as unsatisfactory if the squamo-columnar junction or the transformation zone was not fully visualized (Terminology adopted at the Second World Congress 1975) (121). The transformation zone was considered as atypical if white epithelium, mosaicism,

punctation, keratosis or atypical vascular patterns were seen (93, 149, 161,). When the upper part of the squamo-columnar junction or the transformation zone was not fully visualized, it was manipulated with a cotton-tipped applicator or with a self-retaining Kogan endocervical speculum.

According to the above classification, 118 (83.7 %) of 141 patients examined colposcopically had an atypical transformation zone; in the remaining 23 patients (16.3 %) no atypical lesions were found. In earlier investigations a false negative colposcopic finding has been reported in approximately 10 to 20 per cent of cases with CIN 3 (91, 137). Among those patients with an atypical transformation zone, the upper limits of the lesions were not fully visualized in four patients (3.4 %). This low frequency of unsatisfactory colposcopy is in agreement with the rates in some other investigations (88, 97, 140, 172). In two patients the colposcopic examination showed that the terminal vessels were of irregular size and shape suggestive of invasive carcinoma. As biopsy confirmed the diagnosis of invasive carcinoma, the patients were excluded from this study (see p. 43).

Technique of biopsy. Biopsies were taken without anaesthesia from the area of the most atypical transformation zone on the exocervix using a Schubert biopsy forceps 8 mm x 4.5 mm. If no atypical lesions were colposcopically visible, the biopsies were taken from the area of the transformation zone or from the squamo-columnar junction of the cervix. After four to five punch biopsies were taken from the exocervix, an endocervical curettage including the whole cervical canal was also performed in most cases, using a sharp Récamier curette number five. The tissue specimens were immediately fixed in seven per cent solution of formaldehyde and submitted to the laboratory. No endocervical curettage was performed in four patients of 5 to 15 weeks' gestation at the time of examination. Complications of biopsies occurred only in one patient, in whom bleeding necessitated one day's stay in the hospital.

Table 22 summarizes the methods of diagnostic evaluation in the total material.

Table 22. Diagnostic evaluation of patients in the Period I study (N=201)

Method of evaluation	No. patients	Per cent
Colposcopically directed biopsy and in most cases ECC	141	70.1
Biopsy and ECC and, when indicated, endometrial curettage	57	28.4
Curettage following abortion	3	1.5
Total	201	100.0

c) Histopathological diagnosis

In 10 patients the initial biopsy did not reveal CIN 3 despite the fact that cytologic findings suggested CIN 3. As repeated cytology indicated persistent lesions, the diagnostic biopsy was repeated 4 to 10 months (mean 7 months) later. The histopathological examination revealed CIN 3 at that time, and the patients were eventually included in the study. The location of the CIN 3 lesions in the total material according to the histopathological report is shown in Table 23.

Table 23. Location of CIN 3 lesions according to histopathological report in the Period I study

Location	No. patients	Per cent
Exocervical	163	81.1
Exo-and endocervical	28	13.9
Endocervical	7	3.5
Not identified	3	1.5
Total	201	100.0

The lesions were exocervically located in the majority of the patients. In three patients (1.5 %) the diagnosis was obtained from tissue specimen of squamous epithelium from curettage following spontaneous or legal abortion, and it was thus not possible to determine the location of the lesions. Glandular involvement was found in 92 patients (45.8 %). Of the seven patients with endocervical location of the lesions by histopathology, the squamo-columnar

junction was not fully visualized in one patient, while in another patient the squamo-columnar junction was visible but no focal lesions were detected. Colposcopy was not performed in the five remaining patients with endocervical lesions. Out of 28 patients with combined exo- and endocervical lesions diagnosed by histopathology, 18 patients had atypical colposcopic findings in which the upper limits of the lesions were visible; one had unsatisfactory colposcopy, and in four patients no lesions were visible (colposcopy was omitted in five patients).

3. Cytologic findings following diagnostic biopsy

Following the histopathological diagnosis of CIN 3, the patients were requested to return at 3, 6 and 12 months after the diagnostic procedures for repeat cytology, pelvic examination and, in most cases, colposcopy. Subsequently they were re-examined once a year, providing that all smears were negative. Whenever abnormal cytologic findings appeared, the patients were submitted to cryosurgical treatment. The results of the post-biopsy cytologic examination and the length of follow-up of patients with persistently negative cytologic smears are presented in Table 24.

In all reports on results and length of follow-up throughout the present investigation, Column A in the tables represents the number of patients classified as failures in each period. Those patients with abnormal cytology and/or histopathology were submitted to further evaluation and/or treatment and excluded from the group followed-up during the next interval. Column B represents the number of patients without evidence of persistent or recurrent disease in each period. Furthermore, Column B shows the length of follow-up of patients with persistently negative cytology or histopathology. Column C presents the number of patients not yet followed-up in each period when the investigation was finished in June 1982 (patients lost to follow-up are included in Column C).

Of the 201 patients with a histopathological diagnosis CIN 3, 171 (85.1 %) had abnormal smears identified during the follow-up period. In the first post-biopsy cytologic smear, 145 patients (72.1 %) had abnormal smears, whereas 56 patients (27.9 %) were without evidence of disease. However, during the follow-up period cytologic smears reverted to abnormality in another 26 patients (Table 24).

Table 24. Results of post-biopsy cytologic examination in each period and length of follow-up in patients with persistently negative cytology in the Period I study

	A	B	C
Time after biopsy (in months)	No. patients with abnormal cytology	No. patients with negative cytology	No. patients not followed-up
Less than 6	145	56	0
6 - 11	13	43	0
12 - 23	6	37	0
24 - 35	6	31	0
36 - 47	0	31	0
48 - 59	1	29	1
60 - 71	0	28*	2
72 - 83	0	26	4
84 - 95	0	18	12
96 - 107	0	7	23
108 - 119	0	1	29
120 - 131	0	0	30
Total no. of patients with abnormal cytology	171		

*) One patient with negative cytology had a CIN 3 lesion at biopsy followed by negative cytology and histopathology.

At the end of the examination period (June 1982), 30 patients were still without signs of recurrent disease. Two groups of patients could thus be discriminated on the basis of the cytologic findings some time after the diagnostic biopsy:

a. Patients with persistently negative cytology requiring no further treatment during the study period (N = 30).

b. Patients with abnormal cytologic findings submitted to cryosurgical treatment (N = 171).

These two groups of patients will be discussed separately.

a. Patients with persistently negative cytology requiring no further treatment during the study period

As reported above, 30 patients had no signs of abnormal cytology during the time of follow-up after the diagnostic procedures. No statistically significant difference was found between these 30 patients as contrasted with the 171 patients with abnormal post-biopsy cytologic findings on the following variables: age at first pregnancy, classification of initial smear and first abnormal smear, number of negative and abnormal smears prior to biopsy, time from first negative to first abnormal smear, time from first abnormal smear to biopsy diagnosis of CIN 3 and age at time for diagnosis of CIN 3; nor was there any difference in the frequency of colposcopically directed biopsies. In contrast, the patients with negative post-biopsy cytologic findings had fewer pregnancies ($p < 0.03$), had a less advanced degree of cytologic smear prior to biopsy ($p < 0.01$) - i.e. CIN 2 in 40 % versus 21 %, and CIN 3 in 53 % versus 76 % - less frequently had glandular involvement ($p < 0.001$) and more frequently were without contraception ($p < 0.001$). At the colposcopic examination before biopsy, an atypical transformation zone with the upper limits visible was found in 15 patients, whereas no atypical lesion was found in two patients. In the remaining 13 patients no colposcopy was performed. The location of the lesions by the histopathological report was exocervical in 23 patients, both exo- and endocervical in three patients, endocervical in two patients and not identified in two of the 30 patients with negative post-biopsy cytology. The duration of the observation period for patients with persistently negative cytology after the diagnostic biopsy is presented in Table 24; thus 28 patients (93.3 %) were followed-up more than five years. The average time of observation was 7.0 years (range 3.2 - 9.3 years). The mean number of negative cytologic smears in the individual patient was 7.4 (range 4 - 12); five or more negative smears were obtained in 96.7 per cent of the patients.

Special histopathological follow-up study

Of the 30 patients with normal cytologic smears after biopsy 25 (83.3 %) were subjected to a special histopathological follow-up study comprising multiple biopsies (at least five punch biopsies) from the transformation zone and an endocervical curettage. This procedure was performed at from 17 to 88 months (mean 48 months) after the diagnostic biopsy. No signs of residual disease were found in 22 patients. In two patients a CIN 1 lesion was histopathologically detected which nevertheless required no further treatment, as the

subsequent cytologic smears were negative. In the remaining patient, a diagnosis of CIN 3 of the exocervix was obtained at the biopsy and ECC performed 5.6 years after the initial diagnostic procedures. The findings at colposcopic examination were considered to be normal, and four previous cytologic smears were negative despite the histopathological diagnosis of CIN 3. Five subsequent smears were without evidence of disease, repeated evaluation by biopsy and ECC did not reveal any signs of persistent disease, and no further treatment was required.

Pregnancies after biopsy in patients with persistently negative post-biopsy cytologic smears

In two women the initial diagnosis of CIN 3 was obtained from tissue specimen at exaeresis for spontaneous abortion. Both women had abnormal smears before pregnancy. There were 19 pregnancies subsequent to the diagnostic biopsy, and their outcomes are shown in Table 25.

Table 25. Pregnancy outcome in patients with persistently negative post-biopsy cytologic smears in the Period I study

Pregnancy outcome	No. patients
Full-term pregnancy	12
Pre-term delivery	2
Spontaneous abortion (at < 16 weeks)	1
Legal abortion (at < 12 weeks)	3
Ectopic pregnancy	1
Total no. of patients	19

The interval from biopsy to conception, as calculated from last menstrual period (LMP), varied from 17 days to 6.4 years. The two pre-term deliveries took place at 32 and 35 weeks' gestation, respectively. Both infants were live and normal at birth.

Comments

Disappearance of CIN lesions following diagnostic biopsy has been reported by many investigators (40, 132, 154). The present findings that 14.9 per cent of the patients with the initial diagnosis of CIN 3 had negative cytology subsequent to the diagnostic biopsy is in good agreement with an earlier investigation (163). In Tronstad & Kirschner's study (1980) where multiple biopsies

were performed from the exocervix together with an ECC under general anaesthesia, the disease was eradicated in 45 per cent of the patients with a diagnosis of CIN 3 during an average follow-up time of 18 months (range 6 - 42 months) (167). In another study of selected cases with a small atypical transformation zone, cervical cytology reverted essentially to normal in 61 per cent following colposcopically directed biopsies (3). However, the follow-up period was limited to less than one year in about one third of the patients in that study.

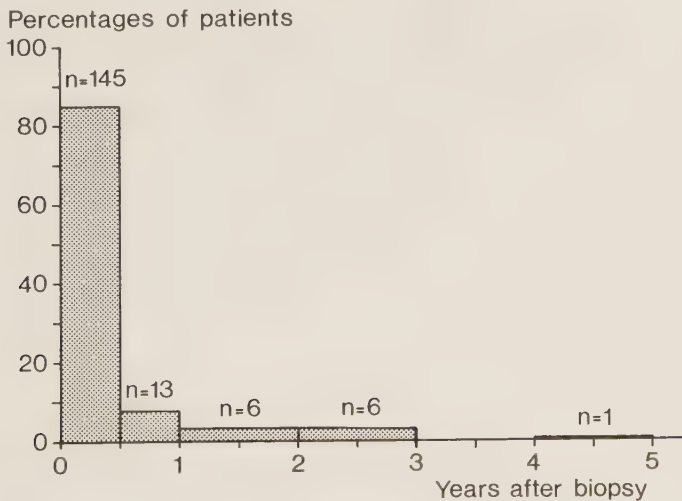
b. Patients with abnormal cytologic findings submitted to cryosurgical treatment

As presented in Table 24, 171 patients with evidence of persistent or recurrent disease were detected by cytologic examination after diagnostic biopsy. These patients constitute the basis for the present study of the effect of cryosurgical treatment.

Interval between biopsy and first abnormal smear

The interval between biopsy and the first abnormal smear is shown in Fig. 7.

Fig. 7. Interval between biopsy and first abnormal smear in the Period I study. Percentages of patients with abnormal smear (N = 171)

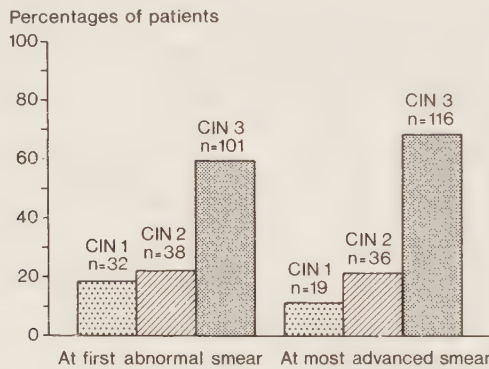


The diagnosis of abnormality appeared at the first smear after the biopsy in 84.8 per cent of the patients and within the first year in 92.4 per cent. All patients with recurrences were detected within five years after the biopsy.

Classification of abnormal smears

The first abnormal smear was classified as CIN 1 in 32 patients (18.7 %), as CIN 2 in 38 patients (22.2 %) and as CIN 3 in 101 patients (59.1 %). The patients with CIN 3 were referred to cryosurgical treatment immediately after the first abnormal smear, while patients with CIN 1 and 2 were subjected to repeated smears before treatment. If pregnancy occurred, treatment was postponed and a repeated smear was taken after delivery or abortion. In 15 patients, the smear changed from a lower degree of dysplasia to CIN 3 during the time of observation. Fig. 8 shows the distribution of different degrees of CIN in the first abnormal smear and in the most advanced smear taken before treatment, respectively.

Fig. 8. Percentages of patients with different degrees of CIN at the first abnormal smear and at the most advanced smear, respectively, in the Period I study



Repeated diagnostic evaluation could be performed in 11 of those 13 patients with an observation time of more than one year from the time of diagnosis to the detection of recurrent disease. The purpose of this was to exclude the possibility that progression to invasive carcinoma had occurred. In each patient colposcopy showed an atypical transformation zone, and cervical biopsy and ECC revealed CIN 3. As this diagnostic procedure might have eradicated the disease, a repeated cytologic smear was taken after three months. The smear disclosed CIN 2 in four patients and CIN 3 in four patients. One patient, who had negative cytologic smears during the first year of observation after the second biopsy, had a third biopsy taken from an atypical transformation zone, this last biopsy indicating CIN 3. In the remaining two patients, the biopsies were taken in connection with treatment. In the three latter patients, no pre-treatment cytologic smear was obtained but colposcopic examination at the time of treatment indicated persistent disease.

Pregnancies between diagnostic biopsy and cryosurgical treatment

At the time of the initial diagnostic biopsy, four patients were pregnant and subsequently delivered uneventfully. In addition 13 pregnancies occurred in 12 patients during the time interval. The outcomes of these pregnancies are shown in Table 26.

Table 26. Pregnancy outcome between diagnostic biopsy and first cryosurgical treatment in the Period I study

Pregnancy outcome	No. pregnancies
Full-term pregnancy	10
Spontaneous abortion (at < 16 weeks)	3
Legal abortion (at < 12 weeks)	3
Ectopic pregnancy	1
Total no. of pregnancies	17

A persistent or more advanced degree of CIN was discovered after delivery or abortion in 15 patients, while one patient with negative smears after biopsy revealed abnormal smears during pregnancy. The suggestions in earlier reports that the trauma of delivery might eradicate the disease was thus not confirmed in the present investigation (42, 146).

4. Cryosurgical treatment and clinical precautions in connection with treatment

Immediately before cryosurgery was performed, a history was always taken with emphasis upon salpingitis or other gynaecologic disorders during the three months prior to treatment. The date of the last menstrual period and the current contraceptive method were recorded. An attempt was made to perform the treatment within one week of the cessation of a menstruation. The patients were always informed that lower abdominal discomfort might occur during the procedure.

a. Method

A standardized technique of freezing was used consistently throughout the study. The procedure was performed on an out-patient basis and without general anaesthesia, analgesia or sedatives. The patient was placed in the lithotomy

position and a self-retaining bivalve vaginal speculum was inserted into the vagina. If the woman requested pain-relief, a local infiltration anaesthesia was given with five ml Carbocaine^(R) adrenalin, 5 mg/ml, in each lateral fornix. After July 1977 (4.5 years after the start of the study), the method of local anaesthesia was changed to 4-5 sprays with Xylocaine^(R) aerosol solution, 10 mg per spray, applied deeply in the vagina. A single-hooked volsellum forceps (Schroeder) was applied to each lateral fornix. The cervix was exposed centrally and cleansed with Klorhexidin^(R) solution 0.05 % (Chlorhexidin. acet. 0.05 g, Natr. acet. 2.1 g, Acid. acet. 5M ad pH 7.9, aq. purif. ad 100). Any intrauterine device which was present was left in place during treatment. The probe was applied at room temperature against the cervix, and the freezing was started with the probe lightly pressed against the cervix by traction of the volsellums. Within 10 to 15 seconds, when the probe adhered to the tissue, the traction was stopped. The duration of freezing was timed from the moment the footswitch of the equipment was released. The freezing time was three minutes, which always caused the ice-ball to extend 4-5 mm beyond the edge of the probe. The first active thawing with the heater lasted three minutes. During the thawing the probe could be released from the tissue at 50-55 seconds, but was kept in contact with the tissue for the full three minutes. After the first thawing the tissue was refrozen in the same way during three minutes, followed by active thawing until the probe could be released at 50-55 seconds.

Following treatment, the patient was allowed to get up but stayed in the Department for observation for 20 minutes. The patient was informed about the possibility of heavy and sometimes bloody discharge. No prophylactic local or general treatment was given. Due to unpredictable vaso-motor reactions to the treatment, car-driving was not allowed for one hour after treatment. The patient was recommended to abstain from intercourse, tub baths and swimming until the discharge decreased. The use of pads was recommended instead of tampons. If the patient did not have the opportunity to change pads every other hour at work, she was advised to stay home until the discharge diminished. If her temperature exceeded 38°C or if pains or other signs of complications appeared, the patient was advised to seek medical attention. During the first two months following treatment, the patient was asked to record the amount of discharge (estimated roughly by the number of pads used daily), bleeding and any other symptoms possibly related to the treatment.

The TCC 10 system was used with 136 patients (79.5 %), the BMS 40 system with 33 patients (19.3 %), and both systems (in sequence) with two patients (1.2 %). As discussed previously (p.35), the equipment had to be changed when more than two patients were treated in a row to avoid excessive cooling of the cylinder, which resulted in reduction of the gas supply pressure. During the first year and a half of the study, only the TCC 10 unit was available, which restricted each treatment session to two patients in a row. During the treatment some patients reported abdominal pain, but the treatment never had to be interrupted because of this discomfort. The number of patients experiencing pain during the procedure and the methods of anaesthesia used are shown in Table 27.

Table 27. Pain and use of local anaesthesia during the first cryosurgical treatment in the Period I study

Category of local anaesthesia	No. patients	Pain during treatment		
		<u>None</u>	<u>Slight</u>	<u>Severe</u>
		No.	No.	No.
No local anaesthesia	95	86	7	2
Local infiltration	66	63	3	
Spray	10	10		
Total	171	159	10	2

About 93 per cent of the patients felt no pain during treatment. Only two patients had severe pain. Even though an attempt was made to give the treatment close to the end of a menstrual period, this was not possible in all cases, as shown in Table 28.

Table 28. Day of menstrual cycle at time of first cryosurgical treatment in the Period I study

Day of cycle	No. patients	Per cent
1- 6	28	16.4
7-13	85	49.7
14-20	27	15.8
21-28	10	5.8
Pregnancy (6 weeks)	1	0.6
Amenorrhoea	20	11.7
Total	171	100.0

Among the patients with a known menstrual history, the treatment was performed in the proliferative phase in 75 per cent of the patients and in the secretory phase in 25 per cent. The treatment was performed during amenorrhea in 20 patients (12 %), mainly after pregnancy. One patient with a history of oligomenorrhea and infertility had an undiagnosed early pregnancy at the time of freezing. This pregnancy had an uneventful course and resulted in a normal delivery at term. The methods of contraception used by the patients at the time of treatment are summarized in Table 29.

Table 29. Method of contraception at time of first cryosurgical treatment in the Period I study

Method	No. patients	Per cent
Oral contraceptives	75	43.9
Barrier method	33	19.3
IUD (in situ)	15	8.8
IUD (removed prior to treatment)	2	1.2
No contraceptive	44	25.6
Unknown	2	1.2
Total	171	100.0

Comments

Out-patient treatment has been used in most other studies. In these studies patients were admitted to hospital only if treatment was performed prior to planned operation (71) or before sufficient clinical experience was obtained with cryosurgery (10). Light analgesia or paracervical block anaesthesia has been used in some cases (34, 70, 153), but in most studies no anaesthesia was given. The proliferative phase of the menstrual cycle has usually been selected for treatment in order to avoid freezing in a pregnant patient and to prevent temporary cervical obstruction and haematometra by the post-freeze oedema (10, 40, 160, 162). Pregnancy has been mentioned as contraindication for cryotherapy, though adverse effects on the child have not been demonstrated thus far (33, 34). The outcomes of pregnancies in a series of seven women treated at 6 to 30 gestational weeks were reported completely normal in five cases; a pre-term delivery occurred in one case, while the remaining case had a spontaneous abortion five weeks after treatment (131). Any intrauterine contraceptive device left in place during treatment have in earlier studies

been reported not to influence the healing (32, 40, 168), nor were the threads of the IUD affected (161). Oral contraceptives and local contraceptive methods had no effect upon the healing after cryosurgery (161, 162).

b. Side-effects and complications of cryosurgical treatment

Various side-effects and complications of cryosurgical treatment have been reported in the literature. These can be divided into the following categories:

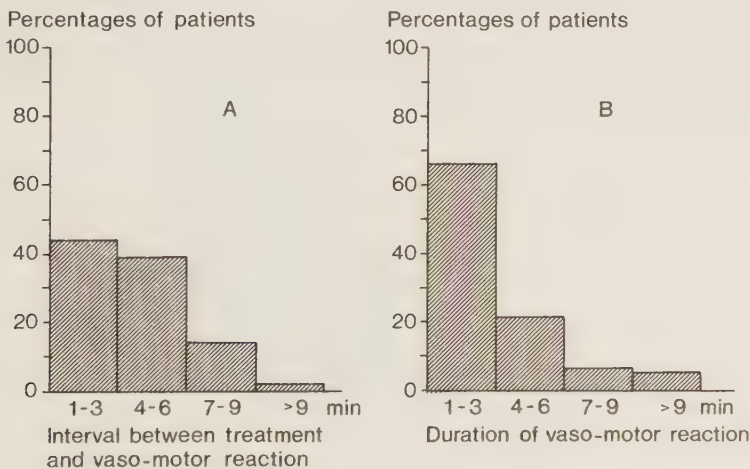
1. Vaso-motor reactions (e.g flush, headache, dizziness).
2. Local effects (e.g. discharge, bleeding, pain).
3. General effects (e.g. weakness, fever, electrolytic changes).

The occurrence of these side-effects and complications was found to be as follows in the present study:

1. Vaso-motor reactions

Reports of the occurrence of vaso-motor reactions were obtained in 157 (91.8 %) of the 171 patients treated. Within a few minutes after the second freezing, 77 patients (49.0 %) experienced facial hot flushes. Immediately preceding the patient's report of this symptom, a red or sometimes cyanotic colour could be observed in the face, upper trunk and upper extremities. The flush appeared at a mean of 4.3 minutes (range 1-10 min) after the second freezing. The duration of the symptoms and signs are shown in Fig. 9.

Fig. 9. Vaso-motor reaction after cryosurgery (N=77). (A) Interval between treatment and appearance of vaso-motor reaction. (B) Duration of vaso-motor reaction.



No vaso-motor reaction occurred in 80 patients (51.0 %). Seven patients (4.5 %) reported headache, dizziness, nausea and a feeling of tension in the temporal region in connection with the vaso-motor reaction. These other symptoms appeared 5 to 30 minutes after the end of treatment and had a duration varying from two minutes and two hours. One patient felt obstruction of her nose appearing eight minutes after treatment and lasting for four minutes.

Comments

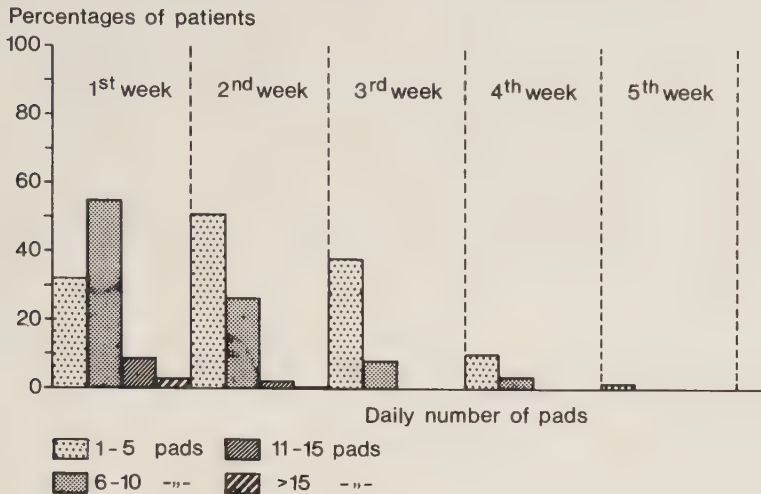
Vaso-motor reactions following cryosurgery have been reported to occur in 10 to 80 per cent of the patients receiving treatment (7, 148, 160, 163, 167). Vaso-vagal attacks combined with syncope and short loss of consciousness have been reported in a few cases (7, 17, 130, 148, 160, 168, 169). The symptoms described are thought to be caused by the release of histamine secondary to necrosis of the cells (34). An increased amount of histamine and methyl-histamine has been found in the urine some hours after cryosurgery in women given standardized histamine-limited food prior to treatment (166).

2. Local effects

The most common local side-effect was a vaginal discharge starting a few hours after treatment, reaching a maximum within an average of one week, and then gradually subsiding. The discharge was clear, water-like or slightly yellowish during the first two weeks, thereafter changing to a more mucoid appearance, the discharge persisting for an additional three weeks. The patients were asked to record the number of pads used daily in order to estimate roughly the amount of the discharge.

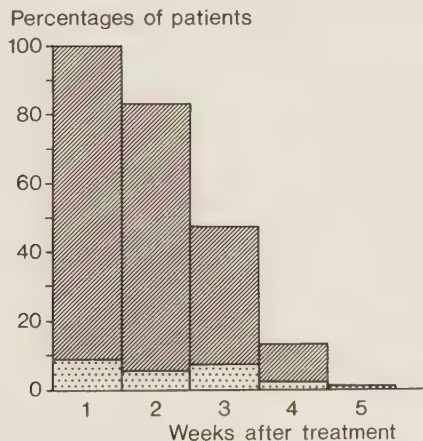
Information about the discharge and other local effects was obtained for 163 (95.3 %) of the 171 patients treated. The number of pads used daily per patient from one to five weeks after treatment is shown in Fig 10. The amount of discharge was most pronounced during the first two weeks.

Fig. 10. Percentages of patients with vaginal discharge estimated by the daily number of pads used one to five weeks following treatment



Bloody discharge appeared in 41 (25.2 %) of the 163 patients who kept a record of the discharge. Normal menstruation at the expected time was not included, nor was the pink-tinged discharge during the first hours after treatment which is due to the inevitable slight bleeding from the application site of the volsellum forceps. The percentages of patients with vaginal discharge, including bloody discharge during the five weeks after treatment are shown in Fig 11.

Fig. 11. Percentages of patients with vaginal discharge of various duration after treatment (1-5 weeks), and percentages with bloody discharge (dotted area)



About 50 per cent of the patients had vaginal discharge for more than two weeks after treatment. In addition to the above-mentioned appearance of bloody discharge, a delayed bleeding appeared in one patient five weeks after treatment and lasted for four weeks. The patient had a von Willebrand's haemorrhagic disease. One patient was admitted to the hospital 17 days after treatment because of heavy bleeding on the expected date of menstruation. The initial haemoglobin concentration of 12.2 g/l decreased to a minimum of 10.5 g/l five days later. Peroral iron treatment was sufficient. The frequency of bloody discharge and the relationship between time of cryosurgery and a known menstrual cycle are shown in Table 30.

Table 30. Frequency of bloody discharge in relation to the time of cryosurgery in a known menstrual cycle (N=150)

Day of cycle	Treated patients	Patients with bloody discharge	
	No.	No.	Per cent
1 - 6	28	5	17.9
7 - 13	85	20	23.5
14 - 20	27	7	25.9
21 - 28	10	6	60.0

The highest frequency of bloody discharge (60.0 %) occurred in patients who underwent premenstrual cyrosurgery. Malodorous discharge was reported by 49 patients (30.1 %) during the first two weeks after treatment. Its occurrence was independent of the time at which treatment took place in the menstrual cycle. Contact-bleeding during intercourse was reported by eight patients (4.9 %) in the period three to five weeks after treatment. Menstrual-like lumbar pain was recorded by five patients (3.1 %) the first two days after treatment. One of these patients had an IUD in situ during treatment (15 had IUD in situ at treatment). Analgesic was sufficient to alleviate the discomfort. No symptoms referred to the bladder or rectum were reported. Late local complication in terms of a reduction of the diameter of the cervical canal to less than six mm was found in four patients. These patients had no complaints, and the reduction was incidentally detected at a cervical curettage between one and seven years after treatment. In a total of 107 patients, the cervical canal was measured with a Hegar dilator in connection with a curettage. The diameter of the cervical canal found in this series is shown in Table 31.

Table 31. Diameter of the cervical canal measured by Hegar dilator in 107 patients one to seven years after cryosurgery

Diameter of cervical canal		
(in mm)	No. patients	Per cent
4	2	1.9
5	2	1.9
6	41	38.3
≥7	62	57.9
Total	107	100.0

Comments

In all previous studies a heavy, water-like discharge has been reported as always occurring, although the duration may vary (71, 116, 160). Heavy haemorrhage has been described in a few cases (27), while spotting or pink-coloured discharge occurs more frequently; even as high a figure as 55 % has been reported (27, 160, 162). Menstrual-like pain in lower abdomen during the first two days after treatment has been reported in about half of the patients treated (160). In the present series this frequency was only three per cent. Blockage of the cervix a few days after treatment due to necrotic tissue has been reported occasionally (56, 159) but was not observed in the present study. Reduction of the cervical canal and a few cases of cervical stenosis have been described in earlier reports (35, 37, 153, 169).

3. General effects

Reports of general side-effects or complications were obtained in 163 (95.3 %) of the 171 patients treated. A general weakness was experienced by 35 patients (21.5 %); 26 of them (74.2 %) reported this during the first week, seven (20.0 %) during the second week, one (2.9 %) during the third week and one (2.9 %) during the fourth week after treatment.

Sick-leave. During the first years of the study, before detailed experience with the side-effects of cryosurgical treatment had been obtained, the patients were often advised to stay home from work during one to two weeks after treatment. During the subsequent course of the study, this was considered to be less necessary. The number of days the patients stayed home from work are shown in Table 32.

Table 32. Number of days of sick-leave after the first cryosurgical treatment in the Period I study

Sick-leave		
No. days	No. patients	Per cent
0	113	66.1
1 - 7	28	16.4
8 - 14	25	14.6
>14	1	0.6
Sick-leave at the time of treatment	4	2.3
Total	171	100.0

The majority of the patients (66 %) in the total series did not stay away from work at all. The change in the author's attitude toward advising the patients to stay home from work is illustrated in Table 33.

Table 33. Mean number of days for treatment-related sick-leave among patients diagnosed 1973 through 1975

Sick-leave	
Year of diagnosis	Mean no. of days
1973	3.8
1974	2.4
1975	1.9
Mean no. of days	2.6

The mean number of days for sick-leave was reduced from 3.8 days in patients diagnosed during the first year to 1.9 days in patients diagnosed in the third year of the present series. The reduced sick-leave time did not correlate with any increase in the side-effects reported by the patients. Four patients reported an increase in body temperature to 38°C - 39°C one to three days after treatment and lasting for one or two days. Antibiotic treatment was not required, and only acetyl-salicylic acid was given. One patient had normal morning temperature but 38°C in the afternoon between the third and the tenth day after treatment, this combined with lumbar pain. No signs of pelvic inflammatory disease were found in these patients with fever. Four patients were admitted to the hospital from eight months to five years after therapy for symptoms suggestive of acute salpingitis. Laparoscopy revealed acute PID

in two of them, one of these subsequent to hysterosalpingography eight months after cryosurgery. No patients with a previous history of PID (14 %) developed an acute salpingitis after treatment.

To study the possible systemic effects of cryosurgery, an investigation of electrolytes and certain other biochemical components of blood and urin was performed in a pilot study of ten patients (Elmfors, Berg and Tryding, unpublished data, 1975). Blood was sampled immediately before treatment, and at 24 hours, 5 days and 11 days after treatment. Twenty-four hour urine samples were collected the day before and the day after treatment and on the fourth and the fifth day. The following blood components were analysed: S-Na, S-K, S-Ca and S-Creatinin. The analysis of urine included quantitative urinary sediment including casts, U-haemoglobin (Sangurtest), dU-Potassium, U-Albumin, U-Density, and U-Osmolality. No notable effects on any of these components were found following cryosurgery.

Comments

A feeling of general weakness following cryosurgery has occasionally been reported in the literature and is considered to be the result of loss of potassium secondary to cellular destruction (26, 27, 34). Collins (1967, 1972) did not find any change in serum potassium but found a high concentration of potassium in the vaginal fluid (26, 27). No electrolytic changes were found in the present study. An increase in body temperature after treatment observed in the current study, has not been reported previously. Some studies have reported acute salpingitis as a complication of cryosurgery (10, 32, 96, 130), and a history of salpingitis has been considered as a contraindication for cryosurgery (153). However, while opinion differs regarding that question, the general view is that treatment should not be performed in the acute stage of a PID (40, 148). A rare case of complication of cryosurgery due to a per-operative accident requiring laparotomy was reported by Levin in 1975 (106).

E. Results of the Period I study *

1. Results of the first cryosurgical treatment

In accordance with the design of the study (p. 37), the patients were followed-up at 3, 6 and 12 months after cryosurgical treatment and once a year thereafter, providing that all cytologic smears and histopathological examinations were without evidence of disease. The long-term results of the first

* For schematic survey of results see p. 137.

cryosurgical treatment are presented in Table 34.

Table 34. Results of the first cryosurgical treatment (N=171) and length of follow-up in patients with negative cytology and histopathology in the Period I study

Time after treatment (in months)	A	B	C
	No. patients with failure	No. patients with negative cytology and histopathology	No. patients not followed-up
Less than 6	26	145	0
6 - 11	5	140	0
12 - 23	6	134	0
24 - 35	2	131	1
36 - 47	1	129	2
48 - 59	0	123	8
60 - 71	0	111	20*)
72 - 83	0	77	54
84 - 95	0	33	98**)
96 -107	0	14	117
108 -119	0	0	131
Total no. of patients with failure	40		

*) One patient died of carcinoma of the ovary 66 months after treatment.

**) One patient died of carcinoma of the breast 92 months after treatment.

Column A shows the number of patients with failure in each period, Column B the number and length of follow-up of patients with negative cytology and histopathology and Column C the number of patients not yet followed-up, including patients lost for follow-up. As shown in Table 34, the follow-up period varies. However, at the first check-up less than six months after treatment, 145 (84.8 %) of the 171 patients treated had normal smears, whereas 140 (96.6 %) of the remaining 145 patients were without signs of persistent disease at the end of the first check-up year. At the end of the second check-up year, 134 (95.7 %) of these 140 patients still had negative cytologic smears. All patients could be followed-up for a minimum of 12 - 23 months after treatment. After that time a decreasing number of patients could be followed-up in each period; thus during the third year after treatment, 133 patients were followed-up, 131 (98.5 %) of them had no signs of recurrent disease. During the

fourth year, 130 patients were followed-up, 129 (99.2 %) of them were without signs of disease. No patients with recurrence were detected after the end of the fourth year after treatment (among those followed-up after that time). Two patients died of intercurrent disease during the follow-up period.

The mean observation time for the 131 patients without signs of persistent disease was 6.1 years (range 1.6 - 8.7 years); 84.7 per cent were followed-up more than five years. The mean number of negative cytologic smears in the individual patient was 7.3. A majority of the patients (94.7 %) had five or more negative smears. All smears were negative in 101 patients, while the first cytologic smear after treatment was classified as abnormal in 30 patients. In 17 of these 30 patients, the first smear was taken less than three months after treatment (mean 84 days). As so-called repair cells during the healing following treatment can be confused with dysplastic cells (17, 34, 35, 61, 62, 68, 148, 160, 171), a repeat smear was taken within about one month after the first smear. This check-smear was found to be negative in all patients. The remaining 13 patients with abnormal smear were subjected to cervical biopsy and ECC. CIN 1 was diagnosed in one of these patients. However, subsequent cytologic smears were negative in all 13 patients. As seen in Table 34, there were 40 patients with failure detected during the period of observation. They were referred to further treatment and will be analysed below.

a. Special histopathological follow-up study

According to the design (p. 37), a special histopathological follow-up study was performed at a time ranging from about one year to five years or more after the initial treatment. This special study comprised 127 patients belonging to the group of 131 patients without evidence of disease when the investigation was finished (June 1982). The study included colposcopy, extensive cervical biopsy, ECC and, when indicated because of abnormal bleeding, an endometrial curettage. The examination was performed within three years after treatment in a majority of the patients (Table 35).

In 91 of the patients, colposcopy was performed within one month prior to the biopsy and ECC. The upper limits of the transformation zone were visible in 68 patients (74.7 %), while the transformation zone extended into the cervical canal in 21 patients (23.1 %). The transformation zone was considered to be abnormal in two patients (2.2 %). However, the histopathological examination showed no signs of persistent disease in any of these 127 patients. A complete

examination of the cervix could be performed in three of these patients 2.7 - 4.7 years after treatment, as a total hysterectomy was performed because of other gynaecological disorders. No evidence of cervical abnormality was found in the hysterectomy specimens. Another four patients with repeated negative cytologic smears were included in the special histopathological follow-up study. As CIN 3 lesions were detected in the biopsy specimens, the patients were classified as failures. They are presented in detail in Table 36, p. 76.

Table 35. Interval between treatment and special histopathological follow-up study in the Period I study

Time after treatment (in years)	No. patients
- 1	10
1 - 2	57
2 - 3	24
3 - 4	23
4 - 5	10
> 5	3
Total	127

b. Patients with abnormal findings after the first cryosurgical treatment

As shown in Table 34, a total of 40 of the 171 patients treated were found to have signs of persistent or recurrent disease after treatment. In this report, "persistence" was arbitrarily defined as failure detected within the first 12 months after the therapy, and "recurrence" was, also arbitrarily, defined as failure detected after the first year of treatment. The definition of failure was a cytologic and/or histopathological diagnosis of CIN 2 or 3 or repeated cytologic and/or histopathological findings of CIN 1 at any time during the follow-up period. Thirty-six of the failures were detected by abnormal cytologic smears and four by cervical biopsy and ECC at the special histopathological follow-up study. The interval between treatment and detection of abnormal cytology or histopathology is shown in Table 34. Among the 40 patients with failure, failure was detected within six months in 26 (65.0 %) and within the first year after treatment in 31 patients (77.5 %). Thirty-seven of these patients (92.5 %) had been detected by the end of the second year, 39 patients (97.5 %) by the end of the third year, and all patients with failure were detected by the end of the fourth year. In these 36 patients detected by

abnormal cytology, the smear indicated CIN 1 in five patients, CIN 2 in seven patients and CIN 3 in 24 patients. Patients with cytologic smears indicating CIN 1 had repeated abnormal smears before they were classified as failures. However, even some patients with cytologic findings of CIN 2 had repeated smears; thus before further evaluation and treatment was performed, the cytologic smear was classified as CIN 1 in one patient, CIN 2 in three patients and CIN 3 in 32 patients.

At the special histopathological follow-up study, CIN 3 was detected at biopsy and ECC in four patients despite repeated negative cytologic smears before the biopsy. Since these patients might have special relevance for the discussion of the appropriate manner of post-treatment follow-up, they are presented more in detail in Table 36.

A comparison between the 40 patients classified as failures and the remaining 131 patients without evidence of disease showed that there was no statistically significant difference in age at first pregnancy, number of pregnancies, classification of initial smear and first abnormal smear, number of negative and abnormal smears prior to biopsy, time from first negative to first abnormal smear and time from first abnormal smear to biopsy diagnosis of CIN 3, frequency of colposcopically directed biopsies, glandular involvement or classification of post-biopsy cytologic smear. In addition, there was no statistically significant difference between the TCC 10 system and the BMS 40 system regarding the results, complications or side-effects. The age at time of diagnosis tended to be higher ($p < 0.06$) in patient with failure versus patients without signs of disease after treatment.

At the colposcopic examination before treatment of the 40 patients with failure, an atypical transformation zone with the upper limits visible was found in 27 patients, whereas no atypical lesion was found in four patients. In the remaining nine patients no colposcopy was performed. The location of the lesions by the histopathological report was exocervical in 37 patients, both exo- and endocervical in two patients and endocervical in the remaining patient. The distribution of failures in relation to the age at the histopathological diagnosis is shown in Table 37.

Table 36. Review of the four patients (A-D) with a histopathological diagnosis of CIN 3 despite negative cytology after the first cryosurgical treatment in the Period I study

Location of lesions by histopathology		Time: cryosurgery- biopsy (in months)	No. negative post- cryosurgical smears	Further treatment
Before cryosurgery	After cryosurgery			
A. Exocervical	Exocervical	14	3	Conization
B. Exocervical with glandular involvement	Cervical polyp	14	3	Second cryosurgery
C. Exocervical	Endocervical with glandular involvement	27	4	Cytologic follow-up
D. Endocervical	Endocervical	47	8	Conization

Table 37. Distribution of failures by age at the histopathological diagnosis of CIN 3 in the Period I study

Age	No. treated	No. failures	Per cent failures in each age group
- 19	2	0	0
20 - 24	28	4	14.3
25 - 29	72	13	18.1
30 - 34	41	13	31.7
35 - 39	28	10	35.7
Total no. of patients	171	40	

Of the patients with failure, 42.5 cent were below 30 years of age and 57.5 per cent 30 years or older at the diagnosis of CIN 3. The frequency of patients with failure below 30 years of age was statistically significantly lower than in patients 30 years or older ($p < 0.01$).

2. Results of further treatment of patients classified as failures after the initial cryosurgical treatment

The subsequent management of the 40 patients classified as failures varied.

Table 38. Further treatment of patients classified as failures after the first cryosurgical treatment in the Period I study

Method of treatment	No. patients
Second cryosurgical treatment	22
Conization	14
Biopsy and cytologic follow-up	3
Total hysterectomy	1
Total	40

As seen in Table 38, the main therapeutic methods were a second cryosurgical treatment (55 %) and conization (35 %). The allocation of the patients to the different methods depended on several factors. A thorough discussion of the different therapeutic modalities was made in each individual case, and the patient was given the opportunity to choose the method of treatment. However, patients below 35 years of age were mainly advised to choose a second cryosurgical treatment.

a. Second cryosurgical treatment

A total of 22 patients were found suitable for a second cryotreatment and they accepted the method. The mean age at the second treatment was 31.0 years (range 22.8 - 39.2 years). Nineteen patients (86.4 %) were below 35 years of age. The second cryosurgery was performed directly after the first abnormal smear in 12 patients. The diagnosis of CIN 3 was confirmed by biopsy and ECC followed by abnormal cytologic findings before treatment in nine patients. According to the histopathological examination of these nine patients, the location of the lesions was exocervical in three patients, both exo- and endocervical in two patients and endocervical in four patients. In the remaining patient CIN 3 was diagnosed in a cervical polyp despite three negative cytologic smears before the diagnosis (Table 36, Patient B). Prior to the second cryosurgical treatment, the cytologic smear indicated CIN 2 in one patient and CIN 3 in 21 patients. The second cryosurgical treatment was performed in the same way as the first treatment with the exception that in seven patients an additional freezing procedure was performed using an endocervical cylindric probe (9 mm x 25 mm), also applied with the double-freeze technique. This special cervical probe was used with those patients whose lesions were in the endocervical canal as evident by histopathology or suggested by cytology. The distribution of treatment between different phases of the menstrual cycle was the same as during the first cryotreatment. One patient with an eight-week pregnancy at the treatment underwent a legal abortion four weeks later. The discomfort and side-effects of the treatment experienced by the patients did not show any substantial differences as compared with those during the first cryotreatment. Three of the patients treated with the endocervical probe reported moderate pain. The results of the second cryosurgical treatment and length of follow-up of patients with negative smears are shown in Table 39.

Of the 22 patients submitted to a second cryosurgical treatment, 18 (81.8 %) had persistently negative cytologic smears during a mean observation time of 5.3 years (range 3.6 - 7.1 years) after the second treatment; 72.2 per cent were followed-up more than five years. The mean number of negative cytologic smears in the individual patient was 7.1 (range 6-9). At the special histopathological follow-up study including biopsy, ECC and in most cases colposcopy, performed between one and three years after the second treatment, no signs of disease were found in 17 patients, while CIN 1 was found in one patient but repeated biopsy and ECC was negative.

Table 39. Results of the second cryosurgical treatment (N=22) and length of follow-up in patients with negative cytology in the Period I study

Time after treatment (in months)	A No. patients with failure	B No. patients with negative cytology	C No. patients not followed-up
Less than 6	4	18	0
6 - 11	0	18	0
12 - 23	0	18	0
24 - 35	0	18	0
36 - 47	0	18	0
48 - 59	0	16	2
60 - 71	0	13	5
72 - 83	0	5	13
84 - 95	0	1	17
96 - 107	0	0	18
Total no. of patients with failure	4		

Colposcopic examination was performed in 14 patients after the second treatment. The upper limits of the transformation zone were not visible in 10 patients (71.4 %). The diameter of the cervical canal measured by Hegar dilator was 6 mm or more in 15 patients, 4 mm in two patients and 3 mm in one patient. This last patient was among those treated with the endocervical probe. In the remaining four patients (18.2 %), the first three-month check-up smear indicated CIN 3, thus classifying the patients as failures. These patients were submitted to further treatment.

b. Cold-knife conization

Cold-knife conization was performed in 14 patients after failure of the initial cryosurgical treatment. The mean age at conization was 33.6 years (range 25.8 - 40.3 years). Eight patients (57.1 %) were older than 35 years of age. The cytologic smear prior to conization was classified as CIN 2 in two patients and CIN 3 in ten patients. These patients with abnormal smears were detected at the first three-month check-up after the initial cryosurgical treatment. The remaining two patients had repeated negative cytologic smears after cryosurgery.

However, the special histopathological follow-up study performed with these patients at 14 and 47 months, respectively, after the first cryosurgical treatment revealed CIN 3 in the biopsy specimen (Table 36, Patients A and D). The conization was performed according to a standardized operative technique. The size of the cone-specimen was related to both the age of the patients and the extension of the iodine-negative area of the exocervix. The procedure was completed by curettage of the remaining portion of the cervical canal and the endometrial cavity. The cervical wound was left open. The patients stayed in hospital for an average of 6.7 days (range 3-9 days) and were on sick-leave for an average of 26.2 days (range 7 - 45 days).

The histopathological examination of the cone specimen showed no signs of disease in one patient, CIN 1 in one patient and CIN 3 in 12 patients. Among the 13 patients with persistent disease, the lesions were exocervical in three patients, and both exo- and endocervical in 10 patients. Glandular involvement was found in nine patients. One cone biopsy was not radical in the endocervix and one was neither radical in the exocervix nor in the endocervix.

At the special histopathological follow-up study performed between one and five years after conization, no signs of disease were found in 10 patients. A complete examination of the cervix could be performed in one of these 10 patients 8.2 years after conization as a total hysterectomy was performed because of irregular bleeding. No signs of neoplasia were found in the hysterectomy specimen. In one patient cytologic smears and biopsy at 16 months after conization showed CIN 2. However, all subsequent smears were consistently negative, as was the result of a repeat biopsy and ECC. The follow-up period of those 11 patients not requiring further treatment is shown in Table 40.

The mean observation time was 4.9 years (range 0.2 - 8.3 years); 63.6 per cent were followed-up more than five years after conization. The mean number of negative cytologic smears was 5.2 per case; 54.5 per cent had more than five negative cytologic smears. Three patients (21.4 %) required further treatment. The detection of persistent disease in these cases took place within one year after conization. The histopathological diagnosis of the cone specimen in these cases showed no evidence of disease in one case, CIN 1 in one patient and CIN 3 non-radically removed in one patient (both exo- and endocervically located). These last three patients were submitted to further treatment.

Table 40. Results of conization (N=14) after failure of the first cryosurgical treatment and length of follow-up in patients with negative cytology in the Period I study

Time after conization (in months)	A	B	C
	No. patients with failure	No. patients with negative cytology	No. patients not followed-up
Less than 6	2	12	0
6 - 11	1	9	2
12 - 23	0	9	2
24 - 35	0	8	3
36 - 47	0	8	3
48 - 59	0	8	3
60 - 71	0	7	4
72 - 83	0	5	6
84 - 95	0	3	8
96 - 107	0	2	9
108 - 119	0	0	11
Total no. of patients with failure	3		

c. Biopsy followed by negative cytologic smears

Three patients classified as failures were followed by repeat cytologic smears. After the initial cryosurgical treatment, two of them (25 and 31 years old) had cytologic smears indicating CIN 2 and 3, respectively, within the first year after treatment. One was pregnant at the cryotreatment. At biopsy and ECC the histopathological diagnosis was CIN 3 in both patients. The remaining patient (37 years old) had four negative cytologic smears after the initial cryotreatment. In the special histopathological follow-up study, a diagnosis of CIN 3 was found incidentally at biopsy and ECC (Table 36, Patient C). Following the biopsy and ECC, these patients had 5, 7 and 8 negative cytologic smears during a follow-up period of 3.5, 4.5 and 6.0 years, respectively. Repeated biopsy and ECC, done two to four years after the previous one, showed no signs of persistent disease.

d. Total hysterectomy

A total hysterectomy had to be performed in one patient after the initial cryosurgical treatment for the following special reasons: The patient was 36 years old, V-para, at the time of treatment. The first cytologic smear taken three months after cryotreatment indicated CIN 3. Two months later a non-directed biopsy from the exocervix in connection with a spontaneous abortion showed no signs of disease. After an additional two months, colposcopic examination disclosed a polypoid tumor with enlarged, atypical vessels at the external os. An excisional biopsy revealed a cervical adenocarcinoma, which one month later was treated by a total hysterectomy with adequate vaginal cuff, performed by a combined vaginal and abdominal approach as described by Johnsson in 1974 (81). Histopathological examination of the uterus showed radically removed adenocarcinoma of the uterine cervix. Repeated post-operative examinations during 4.1 years did not reveal any signs of recurrence, and a total of eight vaginal smears were negative. The short interval between freezing and the diagnosis of invasive carcinoma suggests that the tumor could have been present at the time of freezing. At review of the initial biopsy specimen, both squamous cell carcinoma and adenocarcinoma in situ was revealed (see also 109).

Comments

Cases of invasive cervical carcinoma identified after cryosurgery have occasionally been reported in earlier investigations (32, 70, 71, 73, 85, 86, 87, 95, 96, 123, 140), but in these cases the pretreatment evaluation has most likely been unsatisfactory. False negative cytologic smear, misinterpretation of the colposcopic examination, inadequate biopsy, or an error by the pathologist may have led to the false pretreatment diagnosis. Sevin et al. (1979) reported a series of eight patients with invasive cervical carcinoma detected after cryosurgery (144). All but one patient had inappropriate pretreatment evaluation. If an endocervical curettage is not included in the diagnostic procedures, adenocarcinoma of the endocervical canal may be missed (164).

3. Management of persistent disease after two treatments

As mentioned above, persistent disease was found in four patients after two cryosurgical treatments (p. 79) and in three patients after one cryosurgical treatment followed by conization (p. 80). In the four patients with persistent disease after two cryosurgical treatments, the cytologic smear at the first check-up three months after treatment indicated CIN 3. These four patients

were submitted to conization. The mean age at the conization was 30.2 years (range 26.5 - 33.5 years). In the cone specimen a radically removed microinvasive carcinoma (exocervically located) was suspected in one patient. During the 5.8 year follow-up, no evidence of disease was found at cervical biopsy and ECC performed at two times, and eight cytologic smears were negative. In two patients with the histopathological diagnosis of CIN 3 in the cone specimen, radically removed (one with exocervical and one with endocervical location), four and seven negative cytologic smears were found during a follow-up period of 3.0 and 5.1 years, respectively. In the remaining patient, the histopathological diagnosis of the cone specimen was CIN 3, radically removed (endocervical location). However, two post-conization cytologic smears indicated CIN 3; the patient was subjected to a second conization one year after the first one. The histopathological diagnosis of the second cone specimen showed CIN 3, radically removed (endocervical location). Following the second conization the cytologic smears still indicated CIN 3. With consideration for the patient's age (27 years at the second conization) and her desire for further childbearing, a conservative approach was applied. Cervical biopsy and ECC eight months after the second conization showed CIN 1, and all six cytologic smears were negative during a follow-up period of 4.1 years.

The management of the three patients with persistent disease after one cryosurgical treatment and subsequent conization included a second conization in one patient, a total hysterectomy in one patient and a second conization followed by a total hysterectomy in the third patient.

The first patient with the second conization had a histopathological diagnosis of CIN 3 in the first cone specimen, non-radically removed (exo- and endocervical location). The patient was 30 years old at the second conization, performed 22 months after the first one. The histopathological diagnosis of the second cone specimen showed CIN 3, radically removed (exocervical location). The development of post-conization cervical stenosis required repeated cervical dilations. Five cytologic smears were negative during a follow-up period of 4.5 years. Biopsies and ECC at two times showed no evidence of persistent disease.

The patient with a total hysterectomy had no signs of disease in the cone specimen. A post-conization biopsy, ECC and endometrial curettage because of haemorrhage three months after conization revealed the histopathological diagnosis of CIN 3 in the specimen from the endocervical canal. The patient, who was 38 years old and did not want more children, was submitted to a total hysterectomy. The histopathological diagnosis of the hysterectomy specimen was CIN 3 of the endocervical canal. Two post-hysterectomy cytologic smears were negative during a follow-up period of 10 months.

The remaining patient subjected to a second conization followed by a total hysterectomy had a histopathological diagnosis of CIN 1 in the first cone specimen. Repeated post-conization cytologic smears indicated CIN 2 and 3, and the histopathological diagnosis of a biopsy and ECC was consistent with CIN 2. As post-biopsy cytologic smears still indicated CIN 3, a second conization was performed two years after the first one. No signs of persistent disease were found in the second cone specimen. However, the cytologic smears after the second conization still indicated CIN 2, and a histopathological examination of biopsy and ECC one year after the second conization showed CIN 2. The patient, 44 years old, underwent a total hysterectomy. A persistent lesion could not be localized in the hysterectomy specimen. Three subsequent cytologic smears were negative during a follow-up period of 1.6 years.

4. Reproduction after cryosurgery

Within the present series of 171 patients treated with cryosurgery, 61 women had 81 subsequent pregnancies; one woman was already pregnant at the time of the first cryosurgical treatment. The outcomes of the 82 terminated pregnancies included deliveries, abortions and ectopic pregnancies, as shown in Table 41.

Table 41. Pregnancy outcome following cryosurgery in the Period I study

Pregnancy outcome	No. pregnancies
Full-term pregnancy	55
Pre-term delivery	4
Spontaneous abortion (at <16 weeks)	6
Legal abortion (at <12 weeks)	13
Missed abortion (at <16 weeks)	1
Ectopic pregnancy	3
Total no. of pregnancies	82

Forty-nine women had a total of 59 deliveries after cryosurgery: Forty-one had one delivery, six had two deliveries and two had three deliveries. Fifty-six women conceived after one cryosurgical treatment, three women after one cryosurgical treatment and one conization, and two women after two cryosurgical treatments. One woman was pregnant at the time of the second cryosurgical treatment and requested legal abortion. The time interval between the first cryosurgical treatment and conception, as calculated from the last menstrual period (LMP), is shown in Table 42.

Table 42. Interval between first cryosurgical treatment and conception calculated from LMP in the Period I study

Time: cryosurgery-conception	
(in months)	No. pregnancies
Less than 6	14
6 - 11	8
12 - 23	14
24 - 35	16
36 - 47	8
48 - 59	7
≥ 60	14
Total no. of pregnancies	81

Fifty-six of the 59 deliveries were vaginal. Table 43 shows the mean duration of labour of the first vaginal delivery after the cryosurgical treatment in relation to the women's parity before cryosurgery.

Table 43. Mean duration of labour in the first delivery after cryosurgery in relation to parity before cryosurgery (N=46) in the Period I study

Parity before cryosurgery		Mean duration of labour	
		Total duration (hours)	
		Mean	Second stage (minutes)
No. patients		(range)	Mean (range)
0	9	11.9 (2.9-32.1)	15 (1-33)
1	19	6.5 (2.2-16.1)	13 (2-46)
≥ 2	18	5.8 (0.8-18.0)	12 (4-30)
Total	46		

The duration of labour was defined as the time from the occurrence of regular, painful uterine contractions at five-minute intervals to the delivery of the child. The second stage was measured from complete (10 cm) cervical dilation to delivery.

Three cesarean sections were performed: one for abruptio placentae, one for psychosocial reasons and one for premature breech labour. Four pre-term deliveries occurred. The outcomes of the pre-term deliveries are shown in Table 44.

Table 44. Outcome of four pre-term deliveries after cryosurgery in the Period I study

Gestational week	Birth (weight) (g)	Apgar score (at 1/5 min)	Remarks (incl. obstetric history)
30	1,430	7/10	Premature rupture of the membranes. Breech presentation. Cesarean section. History: four full-term pregnancies and three legal abortions before cryosurgery. One full-term pregnancy after cryosurgery.
32	1,690	7/-	Multiple malformations. Death at three days of age. History: no pregnancy before cryosurgery. Two full-term pregnancies with normal infants after cryosurgery.
35	2,430	10/10	History: seven years before cryosurgery delivery at 35 weeks' gestation with birth weight 2,410 g.
36	2,390	10/10	History: four years before cryosurgery one pre-term delivery with birth weight 1,580 g.

All full-term infants had normal Apgar scores (≥ 8) at 1 and 5 minutes with the exception of one infant delivered by vacuum extraction (indication: maternal fatigue) and having Apgar scores of 4 and 6 at 1 and 5 minutes, respectively.

All women currently without contraceptives at the initial cryosurgical treatment were asked about their plans for subsequent pregnancies. Eighteen women reported such plans and 14 of these subsequently became pregnant; 12 delivered normally, one had a spontaneous abortion and one had an ectopic pregnancy. Two of these women conceived within three weeks after the cryosurgical treatment. Five patients in this series had undergone clinical assessment because of infertility of one to six years' duration prior to the cryosurgery. Within two years after treatment, all five patients became pregnant.

Comments

Previous studies have found no evidence of reduced fertility following cryosurgery (10, 33, 35, 40, 70, 71, 88, 130, 160, 163). According to some authors cryosurgery might even have a beneficial effect on fertility (102, 160, 170). In the present series no evidence has been found of any negative effects of cryosurgery on fertility or on the outcome of pregnancy. The data about reproduction presented here must be viewed in relation to the fact that the average age of the patients in the series was 30 years and the mean parity before cryosurgery was 1.7. During the period of this study, the mean parity in Sweden was 0.7 for women between 20 and 29 years of age and 1.8 for women between 30 and 39 years (Quist, J., Statistiska Centralbyrån, personal communication). Thus it might be suggested that the fertility in this series is even somewhat higher than the national average.

II. PERIOD II STUDY

A. Design of the study

The Period II study included the three-year period, 1976 through 1978. The purpose of the investigation during this period was to study the clinical effects of reducing the time interval between the initial abnormal cytologic smear and the cryosurgical treatment, by performing the diagnostic evaluation shortly after the first abnormal smear and, in most cases, by adding cryotreatment to the biopsy. As in the Period I study, patients with a histopathological diagnosis of invasive cervical carcinoma were excluded. Patients referred to diagnostic evaluation had to be of reproductive age with a cytologic smear indicating CIN 1 to 3. The diagnostic procedures included a repeat smear, colposcopic assessment, directed biopsy and ECC. In case of abnormal uterine bleeding, a non-directed biopsy and endocervical and endometrial curettage were performed under general anaesthesia without colposcopic assessment. The patients were managed according to one of three different alternatives, depending on the colposcopic findings:

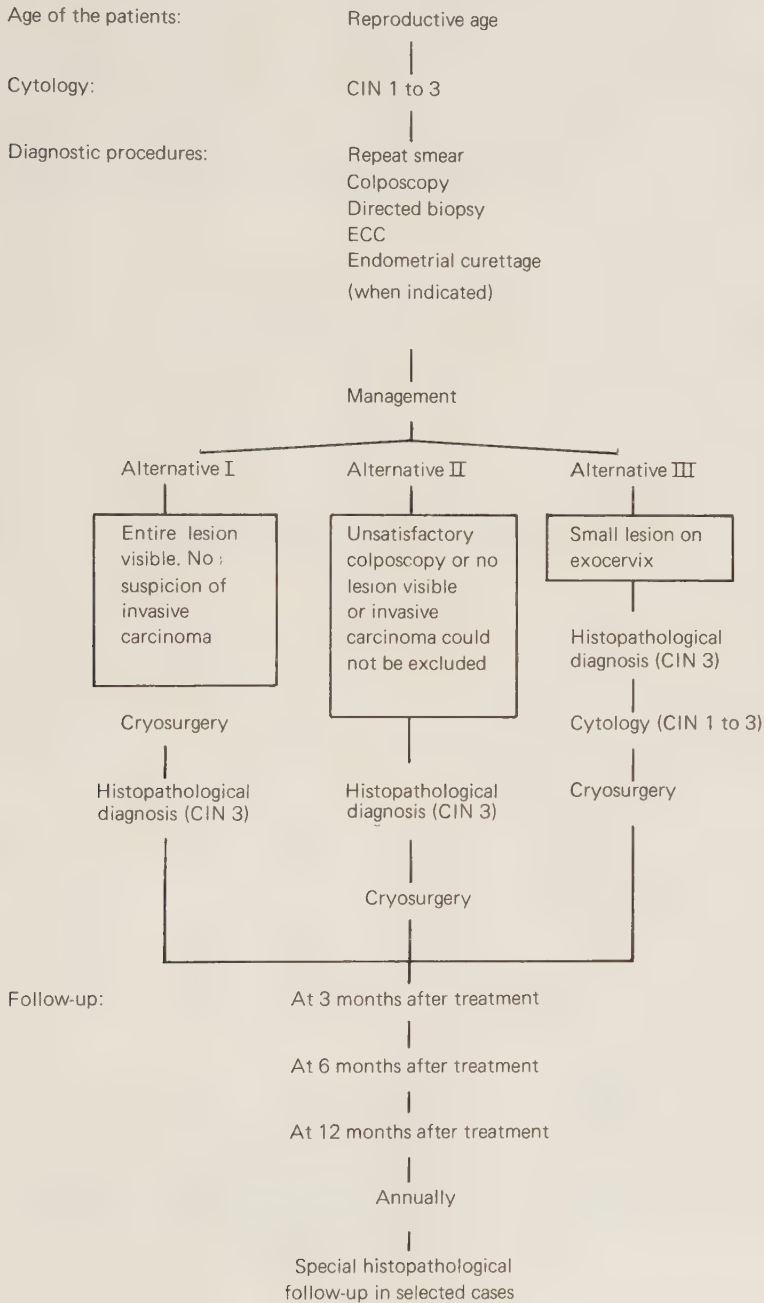
Alternative I: When the upper limits of the lesions were colposcopically visible with no suspicion of invasive carcinoma, cryosurgical treatment was performed immediately after the diagnostic procedures and before the report of the histopathological diagnosis was obtained.

Alternative II: When colposcopic assessment was unsatisfactory or no lesions were seen, or invasive carcinoma could not be excluded, cryosurgical treatment was postponed until the histopathological report was obtained, but no repeat smear was taken. However, in case of unsatisfactory colposcopy in patients 40 years or older, the histopathological diagnosis of CIN 3 led to conization.

Alternative III: When a small lesion confined to the exocervix was found at the colposcopic examination and the lesion's size and nature gave the possibility that the biopsy per se might have a curative effect, the patients were followed by cytology. Only in case of persistent or recurrent abnormal cytologic smears after the diagnostic biopsy were the patients subsequently treated by cryosurgery.

The design of the Period II study is outlined in Fig. 12.

Fig. 12. Design of the Period II study 1976-1978



Those patients in whom colposcopic examination was not carried out were managed in accordance with Alternative II or III. Alternative III was also applied in pregnant patients, with a repeat smear after termination of pregnancy.

The Period II study included only those patients who had a histopathological diagnosis of CIN 3 in the biopsy specimen. Thus, patients with a histopathological diagnosis of CIN 1 and 2, treated by cryosurgery according to Alternative I, were excluded from the study, as were patients without evidence of disease in the tissue specimen.

The technique of cryosurgery was identical to that in the Period I study. Thus, a double-freeze technique with three minutes freezing, three minutes thawing and three minutes freezing was used. After treatment the patients were followed-up at 3, 6 and 12 months and then once a year thereafter, providing all cytologic smears remained negative. The definition of failure and failure rate was the same as that in the Period I study (see p. 37). A special histopathological follow-up study was performed in selected cases (approximately 15 %). Patients with signs of persistent or recurrent disease were treated individually.

Table 45. Number of cases of cervical neoplasia (CIN 3 and invasive carcinoma) diagnosed at the Department of Obstetrics and Gynaecology, Kristianstad, 1976-1978 and incidence rate per 100,000 women and per 100,000 women above 20 years

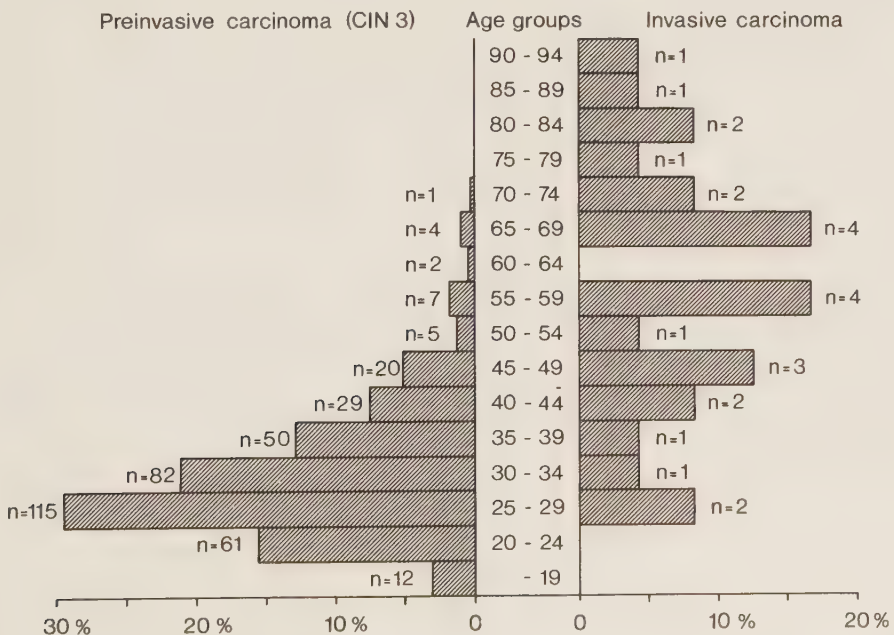
Diagnosis	1976	1977	1978	Total
<u>Preinvasive carcinoma (CIN 3)</u>				
Number of patients	90	166	132	388
Rate per 100,000 women	95.5	174.9	138.3	136.1
Rate per 100,000 women >20 years	126.8	228.0	183.7	180.5
<u>Invasive carcinoma</u>				
Number of patients	9	8	8	25
Rate per 100,000 women	9.5	8.4	8.4	8.8
Rate per 100,000 women >20 years	13.0	11.5	11.4	12.0
Total no. of patients	99	174	140	413

B. Population and general remarks

During the Period II study, the same area was served by the Department of Obstetrics and Gynaecology at the Central Hospital in Kristianstad, as that during the Period I study (see p. 38). At the beginning of the Period II study, the total number of inhabitants of the area was 188,682; the total number of women was 94,190, and the number of women above 20 years of age was 69,140 (Fig. 3, p. 39). The total number of cases of cervical neoplasia (CIN 3 and invasive carcinoma) and the incidence per 100,000 women, as well as per 100,000 women above 20 years of age, identified during the Period II study are shown in Table 45.

The increased number of patients with a histopathological diagnosis of CIN 3 (388 compared with 266 during the Period I study) is probably due to the fact that many of the patients had shown abnormal smears of CIN 1 and 2 when followed for a long time with repeat smears during the Period I study, before the diagnostic procedures of the Period II study eventually were performed. The mean rate of invasive carcinoma in the area was 8.8 per 100,000 women and 12.0 per 100,000 women over 20 years. The age distribution of the patients at the time of the histopathological diagnosis is shown in Fig. 13.

Fig. 13. Percentages of patients with cervical neoplasia (CIN 3 and invasive carcinoma) by age and diagnosis (N=413) in the Period II study



The 388 patients with a diagnosis of CIN 3 had a mean age of 32.5 years (range 17.5 - 73.9 years), and the 25 patients with a diagnosis of invasive cervical carcinoma had a mean age of 58.8 years (range 27.1 - 93.6 years). Of the 25 patients with invasive carcinoma, nine (36.0 %) were below 50 years of age and four (16.0 %) were below 40 years at the time of diagnosis.

C. Patients excluded from the present investigation

As mentioned above, patients with a diagnosis of invasive cervical carcinoma were excluded from the study, as were patients 40 years or older with unsatisfactory colposcopy and patients after menopause. A brief description of the patients who were excluded will be given.

1. Patients with diagnosis of invasive cervical carcinoma Stage I-IV

The following facts of clinical interest are given about the 25 patients with invasive cervical carcinoma: One patient classified as failure after cryosurgery in the Period I study was described in detail on p. 82 and was excluded from the report given below. In 14 patients (58.3 %) the diagnosis was made when the patient sought medical advice due to abnormal vaginal bleeding or heavy vaginal discharge in the menopause. In 10 patients (41.7 %) the diagnosis was made after the discovery of an abnormal cytologic smear taken at routine examination for reasons unrelated to a cervical disorder. One of the patients had undergone a subtotal hysterectomy 22 years prior to the diagnosis of invasive carcinoma.

Cervical cytologic smears were taken in 14 patients prior to or at the time of diagnosis. In 11 of these patients the cytologic smears were abnormal, while in the remaining three patients (21.4 %) no abnormality was found in smears taken up to six months before the diagnosis of invasive carcinoma (two of these three patients had adenocarcinoma).

In 21 patients the diagnosis was based on cervical biopsy or cervical dilation and curettage; in two other patients the diagnosis was confirmed by conization when punch biopsy suggested invasive carcinoma. In the remaining patient the diagnosis of invasive carcinoma was obtained at total hysterectomy after a

histopathological diagnosis of CIN 2 in specimen of cervical curettage. All patients were treated primarily by radiation except one in whom a total hysterectomy alone was performed after a primary conization showed microinvasive carcinoma. Of the three patients below 40 years of age, one had overt carcinoma, while in the remaining two patients invasive carcinoma was found at colposcopic examination and was confirmed by biopsy. The last three patients mentioned had two, two and three cytologic smears suggesting CIN 1 within 3 years, 10 months and 8 months, respectively, before biopsy diagnosis of invasive carcinoma.

Of the 24 patients with invasive carcinoma, nine (37.5 %) died during an observation period of three to six years (range of 2 to 64 months, mean 17 months) after the detection of the disease. The clinical stage of the disease and the histopathological diagnosis of the total number of patients with invasive cervical carcinoma in the Period II study are shown in Table 46.

Table 46. Invasive cervical carcinoma in the Period II study. Clinical stage and histopathological diagnosis. The number of deaths during the observation period given in brackets.

Clinical stage	Total no.	Histopathological diagnosis	
		Squamous carcinoma	Adenocarcinoma
		No. patients	No. patients
I A	5 (1)	4 (1)	1
I B	4 (1)	4 (1)	
II A	5 (2)	5 (2)	
II B	4	2	2
III-IV	7 (5)	6 (4)	1 (1)
Total no. of patients	25 (9)	21 (8)	4 (1)
Per cent		84.0	16.0

2. Patients with a histopathological diagnosis of CIN 3 but excluded from the present study

Of the 388 patients with a histopathological diagnosis of CIN 3, 56 were excluded from the study. Seven of the patients below 40 years of age were excluded for the following reasons: Three had negative cytologic smears after

biopsy, two were conized after a malignant cytologic smear, one was operated on because of uterine prolapse and one moved out of the area. The remaining 49 patients, 40 years or older, were excluded because of unsatisfactory colposcopy or because of being above the age of menopause. The mean age of those patients who were excluded was 48.2 years (range 24.9 - 73.9 years). The diagnostic procedure or the combined diagnostic/therapeutic procedures in these patients are shown in Table 47.

Table 47. Therapeutic procedure or combined diagnostic/therapeutic procedures in 56 patients excluded from the Period II study

Procedure	No. patients	Per cent
Conization after malignant cytologic smear	10	17.9
Conization after biopsy or cervical curettage	28	50.0
Total hysterectomy after malignant cytologic smear	1	1.8
Total hysterectomy after biopsy or cervical curettage	6	10.7
Total hysterectomy after conization	5	8.9
Diagnosis incidentally found at treatment of prolapsus uteri	2	3.6
Biopsy followed by negative cytology	4	7.1
Total	56	100.0

As seen in Table 47, conization became the final treatment in 68 % and a total hysterectomy in 21 % of cases.

D. Patients treated with cryosurgery

1. Present study material

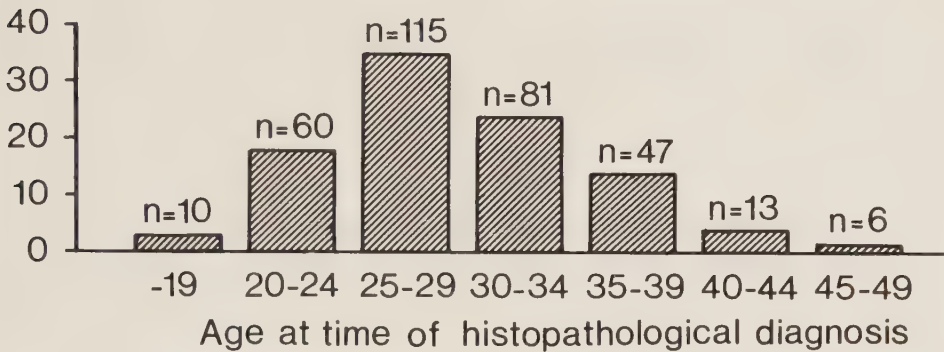
The remaining 332 patients with a histopathological diagnosis of CIN 3 were subjected to cryosurgery and constituted the basis for the present study. Some basic data about the material will be given first.

a. Age distribution

Fig. 14 shows the age distribution of the patients at the time of diagnosis. The mean age was 29.8 years (range 17.5 - 48.1 years). The majority of the patients (55.7 %) were below 30 years of age, while 19 (5.7 %) were 40 years or older.

Fig. 14. Age distribution of patients at time of histopathological diagnosis in the Period II study (N=332)

**Percentages
of patients**



b. Obstetric background data prior to biopsy

Table 48 shows the parity of the patients prior to diagnosis.

Table 48. Parity in 332 patients prior to the biopsy diagnosis of CIN 3 in the Period II study

Parity	No. patients	Per cent	Cumulative per cent
0-para	63	19.0	19.0
1-para	89	26.8	45.8
2-para	114	34.3	80.1
≥3-para	66	19.9	100.0
Total	332	100.0	

The mean parity was 1.6. The number of spontaneous and legal abortions is shown in Table 49.

Table 49. Spontaneous and legal abortions prior to biopsy diagnosis of CIN 3 in the Period II study

No. abortions	Spontaneous abortions	Legal abortions
	No. patients	No. patients
1	35	53
2	9	9
3	1	2
4	1	
Total no. of patients with at least one abortion	46	64

The mean number of pregnancies per patient was 2.1. Fifty-one of the patients (15.4 %) had never been pregnant.

c. Genital infections prior to biopsy

A history of pelvic inflammatory disease was found for 31 patients (9.3 %); eight had had PID twice and two had had PID three times. Thirty-five patients (10.5 %) had a previous history of trichomonas infestation, diagnosed by wet smear technique or by cytology. Twenty-six patients (7.8 %) had a history of gonococcal infection diagnosed by culture; a repeat gonococcal infection was recorded in four patients.

2. Diagnostic approach

a. Cytologic history

The selection of the patients was based on abnormal cytologic smears in 329 patients and on histopathological diagnosis of CIN 3 of specimen from cervical curettage in three cases with previously negative cytologic smears. The reasons for taking the smear at which cytologic abnormality was found are summarized in Table 50.

Twenty-three per cent of the patients with abnormal cytology were detected at gynaecologic check-ups and 15 per cent in connection with pregnancy. All together about 65 per cent of the patients with abnormal smears were identified at check-ups unrelated to any symptoms of disease.

Table 50. Reasons for taking the smear at which cytologic abnormality was found in the Period II study (N=329).

Reasons for taking smear	No. patients	Per cent
Population screening	54	16.4
Routine gynaecologic check-up	23	7.0
Routine obstetric check-up	38	11.6
Request for legal abortion	12	3.6
Contraceptive advice	92	28.0
Complaints unrelated to a cervical disorder	60	18.2
Abnormal uterine bleeding	16	4.9
Other reasons	34	10.3
Total	329	100.0

The cytologic smears were collected in a way identical to that in the Period I study. The same policy was applied in the Period II study as during the Period I study, namely that all patients with negative cytologic smears had a repeat smear taken at a one- to four-year interval (see p. 49). Retrospective analysis of the Period II material showed that the initial smear was negative in 167 patients, while the remaining patients had an initial abnormal smear of a varying degree, as shown in Table 51.

Table 51. Classification of initial smear in 332 patients with a histopathological diagnosis of CIN 3 in the Period II study

Cytology report	No. of cases	Per cent
Negative	167	50.3
CIN 1	62	18.7
CIN 2	60	18.1
CIN 3	43	12.9
Total	332	100.0

The number of patients with one or more negative smears and the average time between the first negative smear and the first abnormal smear is presented in Table 52.

Table 52. Number of patients with one or more negative smears prior to abnormal smear and average time from first negative smear to first abnormal smear in the Period II study

No. negative smears	No. patients	Per cent	Average time from first negative to first abnormal smear (in months)
1	81	49.4	39
2	43	26.2	64
3	25	15.2	88
≥4	15	9.2	96
Total	164	100.0	

As evident in Table 52, three of the patients had never had an abnormal smear but received the diagnosis of CIN 3 at cervical curettage. Table 53 shows the classification of the first abnormal smear and the average time from the first abnormal smear to the biopsy diagnosis of CIN 3 in the total material.

Table 53. Classification of first abnormal smear and average time from first abnormal smear to biopsy diagnosis of CIN 3 in the Period II study (N=329)

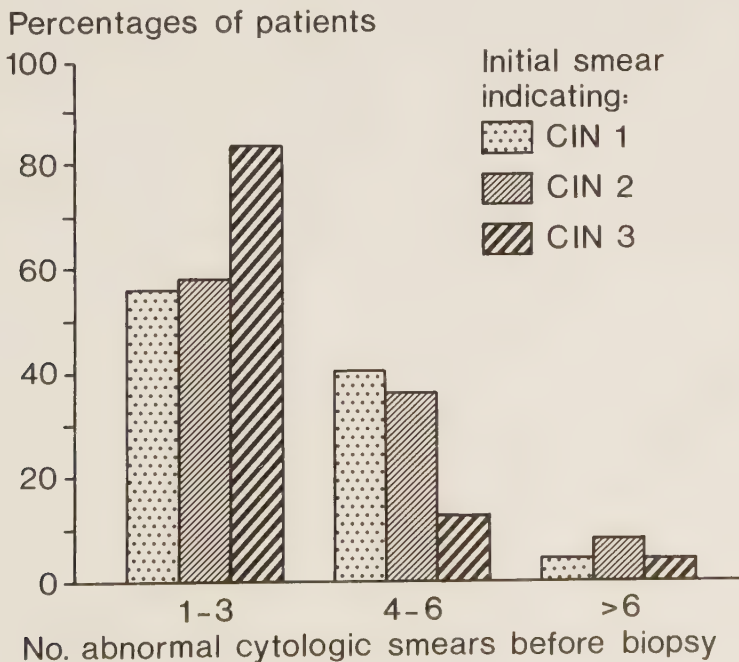
Cytology report	No. patients	Per cent	Average time from first abnormal smear to biopsy diagnosis of CIN 3 (in months)
CIN 1	118	35.9	25
CIN 2	119	36.2	27
CIN 3	92	27.9	13
Total	329	100.0	

As seen in Table 53, the interval between the first abnormal smear and the biopsy diagnosis was about two years in patients with smears indicating CIN 1 and 2 and about one year in patients with smears indicating CIN 3. The reason for this delay is that 130 patients (39.5 %) had their first abnormal smear detected prior to or during the Period I study when repeat smears were taken, in most cases, at six- to twelve-month intervals, until a negative smear was obtained or cytologic verification of CIN 3 had occurred. Thus, by necessity, about one year passed before all the patients could be evaluated according to the design of the Period II study.

In those patients (199 cases) with the first abnormal smear identified during Period II, the mean interval between the first abnormal smear and the biopsy diagnosis was 11, 10 and 6 months when the smear indicated CIN 1 (58 cases), CIN 2 (64 cases) and CIN 3 (77 cases), respectively.

Towards the end of the study, when the design was completely implemented and the study had become well-known to all referring physicians in the area, the time from the first abnormal smear to the biopsy diagnosis was further reduced. Thus during the last year of the study, the average time from the first abnormal smear to the histopathological diagnosis of CIN 3 was 7.7, 5.9 and 3.7 months when smears indicated CIN 1 (13 cases), CIN 2 (23 cases) and CIN 3 (25 cases), respectively. A repeat smear was taken at the first Period II study check-up for patients with a previous history of abnormal smear, and the patients were informed about the three alternatives for management in case a persistent abnormal smear was found. Furthermore, a repeat smear was taken in connection with colposcopy and biopsy. These facts explain why repeat abnormal smears were obtained before the diagnostic evaluation, as shown in Fig. 15.

Fig. 15. Total no. of abnormal smears per patient before biopsy in relation to the cytologic classification of the initial abnormal smear in the Period II study



More than six abnormal cytologic smears were a main characteristic of patients who had moved into the area from other regions of the country. The distribution of the most advanced cytologic abnormality prior to biopsy was that shown in Table 54.

Table 54. Classification of most advanced cytologic abnormality prior to biopsy in the Period II study

Classification	No. patients	Per cent
CIN 1	15	4.6
CIN 2	115	35.0
CIN 3	199	60.4
Total	329	100.0

b. Diagnostic procedures

The diagnostic procedures included a repeat smear, colposcopic assessment of the lesions, colposcopically directed biopsy and ECC. Biopsy, cervical and endometrial curettage were performed under general anaesthesia without colposcopic assessment, when indicated by abnormal bleeding.

Of the 332 patients with a histopathological diagnosis of CIN 3, colposcopic examination was performed in 307 patients (92.5 %). An atypical transformation zone was found in 255 patients (83.1 %), whereas no atypical lesion was found in 52 patients (16.9 %). However, the upper limits of the lesions were not visible in 16 of the 225 patients. Nor were the upper limits of the transformation zone visible in 10 of the 52 patients without atypical findings. Thus, the colposcopic examination was unsatisfactory in 26 patients (8.5 %). Table 55 shows the different methods of diagnostic evaluation of the patients.

Table 55. Diagnostic evaluation of patients in the Period II study (N=332)

Method of evaluation	No. patients	Per cent
Colposcopically directed biopsy and ECC	307	92.5
Biopsy, ECC and endometrial curettage	23	6.9
Curettage following abortion	2	0.6
Total	332	100.0

c. Histopathological diagnosis

Although all the patients during the Period II study fulfilled the criteria of a histopathological diagnosis of CIN 3, some of them also had had a history of biopsy diagnosis of a lower degree prior to inclusion in the study. Thus, in 14 patients with cytologic smears suggesting CIN 2 and 3, the initial biopsy taken after the smear showed no evidence of disease at all in five cases and CIN 1 and 2 in nine cases. As further cytologic smears after the initial biopsy still indicated abnormality, a repeat diagnostic evaluation was performed and revealed CIN 3. In the total material the histopathological diagnosis was CIN 3 in 329 patients and suspected microinvasive carcinoma in three patients. As the microinvasion was not confirmed at further evaluation, these three patients were assessed as CIN 3. The location of the lesions according to the histopathological report is shown in Table 56.

Table 56. Location of CIN 3 lesions according to the histopathological report in the Period II study

Location	No. patients	Per cent
Exocervical	257	77.4
Exo- and endocervical	64	19.3
Endocervical	9	2.7
Not identified	2	0.6
Total	332	100.0

Glandular involvement was found in 115 patients (34.6 %). Of the nine patients with endocervical location of the lesions according to histopathology, the colposcopic findings were as follows: Four patients had an atypical transformation zone with the upper limits of the lesions visible, three patients had an atypical transformation zone with the lesions extending into the endocervical canal and one patient had no focal lesion visible. Colposcopy was not performed in one patient.

3. Cryosurgical treatment

The same standardized technique of freezing as that employed during the Period I study was consistently used throughout the Period II study, i.e. three minutes freezing, three minutes thawing and a repeat three minutes freezing. All patients were treated on an out-patient basis. After July, 1977 (1.5 years after the start of the Period II study), the method of local anaesthesia by infiltration was replaced by spray with Xylocaine(R) aerosol solution. Four of the patients were given general anaesthesia because they strongly preferred examination and treatment under general anaesthesia. The TCC 10 system was used with 269 patients (81.0 %), the BMS 40 system with 62 patients (18.7 %) and both systems (in sequence) with one patient (0.3 %). The treatment was generally performed in the proliferative phase (in 299 of 315 patients with a known menstrual cycle). One patient was treated at nine weeks' gestation (and subsequently delivered normally at term). In no case did the treatment have to be discontinued because of pain. As previously mentioned (see p. 88), the patients were referred to the three different management alternatives for treatment. The distribution of these alternatives is presented in Table 57.

Table 57. Management alternatives for treatment in the Period II study (N=332)

Category	No. patients	Per cent
Alternative I	249	75.0
Alternative II	32	9.6
Alternative III	51	15.4
Total	332	100.0

E. Results of the Period II study *

1. Results of the first cryosurgical treatment

a. Assignment of the patients to the management alternatives

After the cryosurgical treatment, the patients were followed-up at the same intervals as those during the Period I study, i.e. at 3, 6 and 12 months after the treatment and once a year thereafter, providing all smears remained negative. Of the 332 patients included in the Period II study, Alternative I was used with 249 patients, Alternative II with 32 patients and Alternative III with 51 patients. The age distribution of the patients in the three alternative groups at the time of diagnosis is shown in Table 58.

Table 58. Age distribution of the patients at the time of diagnosis by alternatives of management in the Period II study

Age	Alternative of management			Total
	I No.	II No.	III No.	
- 19	8	1	1	10
20 - 24	47	4	9	60
25 - 29	91	6	18	115
30 - 34	56	8	17	81
35 - 39	34	7	6	47
40 - 44	8	5		13
45 - 49	5	1		6
Total	249	32	51	332

About 56 per cent of the total patients were below 30 years and about 6 per cent 40 years or older at the time of diagnosis. The mean ages of the patients managed according to Alternative I, II and III were 29.5, 32.5 and 29.7 years, respectively.

* For schematic survey of results see p. 139.

b. Alternative I management

The cryosurgical treatment was performed in association with the biopsy and ECC in patients with colposcopically visible lesions without suspicion of invasive carcinoma. Those patients presented here are only those (N=249) subsequently verified as having CIN 3 at the histopathological examination of the biopsy specimen. At colposcopy of 247 patients, 203 had an atypical transformation zone, with the upper limits visible in all but one case. The upper limits of the transformation zone were not visible in eight of the remaining 44 patients without atypical findings. The colposcopic report was not available in two patients. According to the design of the Period II study, patients with unsatisfactory colposcopy or not visible lesions were not to be subjected to treatment before the histopathological report was obtained. However, as the study had the goal of reducing the time from the first abnormal smear to the treatment, the design was not always strictly followed, particularly in patients with repeated smears indicating CIN 1 and 2. In addition, cryosurgery was used as a haemostatic treatment when the patients were bleeding from the biopsy sites.

Results of Alternative I management

The results of the first cryosurgical treatment of patients managed according to Alternative I are presented in Table 59. Column A shows the number of patients with abnormal cytology or histopathology in each period, and Column B the number and length of follow-up in patients with negative cytology. Column C presents the number of patients not yet followed-up in each period. One patient moved abroad shortly after treatment and was lost for follow-up. Of the remaining 248 patients, 218 (87.9 %) had negative smears at the first check-up less than six months after treatment. Those patients who showed abnormal cytology or histopathology in each period received further treatment and are thus excluded from the group followed-up in the next period. At the end of the first follow-up year, 211 (97.2 %) of the 217 patients followed-up were still without signs of disease. At the end of the second year, 205 (97.6 %) of the 210 patients followed-up had negative smears. No case of recurrence was detected after the end of the fifth year after treatment among those patients followed-up after that time. All together, 48 patients with failure were detected during the period of observation and were referred to further treatment. The mean observation time for the 200 patients (one lost for follow-up) without evidence of persistent disease was 3.8 years (range 1.0-6.3 years). The mean number of negative cytologic smears in each patient was 4.5.

Table 59. Results of Alternative I management (N=249) and length of follow-up in patients with negative cytology

Time after treatment (in months)	A	B	C
	No. patients with failure	No. patients with negative cytology	No. patients not followed-up
Less than 6	30	218	1
6 - 11	6	211	2
12 - 23	5	205	3
24 - 35	4	193	11
36 - 47	2	165	37
48 - 59	1	102	99
60 - 71	0	32	169
72 - 83	0	5	196
84 - 95	0	0	201
Total no. of patients with failure	48		

c. Alternative II management

In 32 patients cryosurgical treatment was performed when the histopathological report verifying CIN 3 was obtained without a repeat smear. Patients with unsatisfactory or suspected malignant colposcopic findings were included in this group, as well as some patients with a combined biopsy, ECC and endometrial curettage without colposcopy. In 25 patients, the average interval between biopsy and cryosurgery was 53 days. In the remaining seven patients (one pregnant at the biopsy), the treatment was delayed more than three months (mean 120 days) for various reasons. To exclude the possibility that progression to invasive carcinoma had occurred, repeat diagnostic procedures were performed immediately before the cryosurgical treatment in these patients. However, a histopathological diagnosis of CIN 3 remained in all patients.

Results of Alternative II management

The results of the first cryosurgical treatment of patients managed according to Alternative II are presented in Table 60.

Table 60. Results of Alternative II management (N=32) and length of follow-up in patients with negative cytology

	A	B	C
Time after treatment (in months)	No. patients with failure	No. patients with negative cytology	No. patients not followed-up
Less than 6	4	28	0
6 - 11	0	28	0
12 - 23	1	27	0
24 - 35	0	27	0
36 - 47	0	26	1
48 - 59	0	15	12
60 - 71	0	9	18
72 - 83	0	1	26
84 - 95	0	0	27
Total no. of patients with failure	5		

Column A shows the number of patients with abnormal smears in each period, Column B the number and length of follow-up in patients with negative cytology and Column C the number of patients not yet followed-up in each period. Of the 32 patients treated, 28 (87.5 %) were without signs of disease at the first check-up less than six months after treatment as well as at the end of the first post-treatment year. At the end of the second follow-up year, 27 (96.4 %) of the 28 patients followed-up still had negative cytology. No more patients with abnormal cytology were detected during the subsequent follow-up. Five patients were classified as failures and referred to further treatment. The mean observation time for the 27 patients with persistently negative cytologic smears was 4.4 years (range 2.0 - 6.1 years). The mean number of negative smears per patient was 5.3.

d. Alternative III management

The design of the Alternative III management was the same as that of the Period I study, i.e. a repeat smear was taken after the histopathological diagnosis of CIN 3 had been obtained. Patients with a small lesion as well as some patients with a combined biopsy, ECC and endometrial curettage without colposcopy were included in this group. Only those patients who had persistent or recurrent abnormal cytologic smears were submitted to cryosurgery and included in the present study. Among the 51 patients with a histopathological

diagnosis of CIN 3, abnormal smears were found at the first check-up within six months after biopsy in 47 patients (92.2 %). In the period 12 - 23 months after the biopsy, two additional patients with abnormal smears were detected, and in the period 24 - 33 months post-biopsy the remaining two patients had abnormal smears. The most advanced post-biopsy smear was CIN 1 in five patients (9.8 %), CIN 2 in 17 patients (33.3 %) and CIN 3 in 29 patients (56.9 %). Colposcopic examination was performed in 40 patients in connection with the diagnostic procedures and/or before cryosurgery. An atypical transformation zone was found in 36 patients, with the upper limits of the lesions visible in all but one patient. No lesion was visible in four patients. Six patients were pregnant at the initial biopsy and were subjected to a repeat biopsy confirming the diagnosis of persistent CIN 3 after the termination of the pregnancy.

Results of Alternative III management

The results of the first cryosurgical treatment in patients managed according to Alternative III are shown in Table 61.

Table 61. Results of Alternative III management (N=51) and length of follow-up in patients with negative cytology or histopathology

Time after treatment (in months)	A	B	C
	No. patients with failure	No. patients with negative cytology or histopathology	No. patients not followed-up
Less than 6	3	48	0
6 - 11	0	47	1
12 - 23	0	45	3
24 - 35	0	44	4
36 - 47	0	28	20
48 - 59	0	10	38
60 - 71	0	1	47
72 - 83	0	0	48
Total no. of patients with failure	3		

Column A shows the number of patients with abnormal smears in each period and Column B the number and length of follow-up in patients with negative cytology. Column C presents the number of patients not yet followed-up in each period. At the first check-up at less than six months after treatment, three (5.9 %) of 51 patients treated were classified as failures. No more cases with abnormal smear were detected during the period of observation. The mean observation time for the 48 patients with persistently negative cytologic smears was 3.0 years (range 0.2 - 5.4 years). One patient had a hysterectomy performed 10 weeks after treatment because of irregular bleeding in combination with a serious mental disease. CIN 1 was found in the hysterectomy specimen, thus not qualifying the patient as a failure. The mean number of negative cytologic smears per patient was 4.6.

e. Patients with abnormal findings after the first cryosurgical treatment

During follow-up after the first cryosurgical treatment, the patients were classified as failures either when cytology or histology indicated CIN 2 or 3 or when repeated cytologic or histopathological examinations revealed CIN 1 at any time during the follow-up period. Failures appearing within 12 months were classified as persistent disease, and failures appearing later as recurrent disease. As seen in Tables 59, 60 and 61, a total of 56 patients were classified as failures during the total period of observation. The interval between the initial cryosurgical treatment and the detection of persistent or recurrent disease is evident from Table 62.

Table 62. Interval between first cryosurgical treatment and detection of abnormal cytology or histopathology in patients classified as failures (N=56) in the Period II study

Time after treatment (in months)	No. patients with failure	Per cent
Less than 6	37	66.1
6 - 11	6	10.7
12 - 23	6	10.7
24 - 35	4	7.1
36 - 47	2	3.6
48 - 59	1	1.8
Total	56	100.0

Failures were detected by abnormal cytologic smears alone in 55 cases. In the remaining patient, 29 years of age, a diagnostic conization was performed six weeks after treatment because microinvasive carcinoma was suspected at the initial biopsy adjacent to the cryotreatment. In the cone specimen, however, a CIN 3 lesion was found with exo- and endocervical location, radically removed. Of the 55 patients, 11 showed cytologic smears indicating CIN 1 (repeated smears) and 44 showed CIN 2 or 3. Persistent disease was found in 76.8 % of the failures; in the remaining patients recurrence appeared in the two- to five-year follow-up period. After that time no further failures were observed. The distribution of failures in relation to the age at the histopathological diagnosis is shown in Table 63.

Table 63. Distribution of failures by age at the histopathological diagnosis in the Period II study

Age	No. treated	No. failures	Per cent failures in each age group
- 19	10	0	0
20 - 24	60	7	11.7
25 - 29	115	23	20.0
30 - 34	81	14	17.3
35 - 39	47	11	23.4
40 - 44	13	1	7.7
45 - 49	6	0	0
Total no. of patients	332	56	

There was no statistically significant difference in the frequency of failures in patients below 30 years of age compared with patients 30 years or older. The mean age of the patients with failures was 30.3 years (range 20.2 - 40.1 years). A colposcopic examination prior to the initial cryosurgery was performed in 45 of the failure cases. An atypical transformation zone was found in 40 of these 45 patients, the upper limits of the lesions being visible in all but two. No atypical lesion was found in the remaining five patients, the upper limits of the transformation zone being visible in all five cases. The location of the lesions by histopathology was exocervical in 42 patients, both exo- and endocervical in 13 patients and endocervical in one patient. Glandular involvement was found in 29 patients.

A comparison between the 56 patients classified as failures and the remaining 276 patients without evidence of persistent or recurrent disease showed that there was no statistically significant difference on the following variables: age at histopathological diagnosis and the colposcopic findings, including the location of the lesions by colposcopy and by histopathological examination. However, the frequency of glandular involvement was statistically significantly lower ($p < 0.005$) in patients without signs of disease after the first cryosurgical treatment versus patients classified as failures.

2. Results of further treatment of patients classified as failures after the first cryosurgical treatment

The method of further treatment in cases with failure in relation to the initial alternative of management is presented in Table 64.

Table 64. Method of further treatment of patients with failure after the first cryosurgery in the Period II study

Method of further treatment	Initial management			Total
	Alternative I No.	Alternative II No.	Alternative III No.	
Second cryosurgical treatment	33	4	1	38
Conization	14	1	2	17
Total hysterectomy	1			1
Total	48	5	3	56

An attempt was made throughout the present study to reduce the interval between the first abnormal smear and the treatment which resulted in persistently negative cytologic smears. The patients were therefore offered further treatment as soon as they fulfilled the criteria for failure.

a. Second cryosurgical treatment

Of the 56 patients classified as failures, 38 (67.9 %) were submitted to a second cryosurgical treatment. A repeat colposcopy, biopsy and ECC was performed immediately before the second treatment in 33 patients. The colposcopic examination was unsatisfactory in eight patients (24.2 %), atypical lesions were detected in 17 patients (51.6 %), and no lesion was found in eight

patients (24.2 %). The histopathological examination showed no lesion in six patients, CIN 1 in six patients and CIN 2 or 3 in 21 patients. It should be observed that even if no lesion or CIN 1 was found in the biopsy specimen after the initial cryosurgical treatment, the patients were included in the group as a consequence of the abnormal post-treatment cytologic findings. The location of the lesions in those patients (N=27) with persistent disease was exocervical in 14 cases, both exo- and endocervical in four cases, endocervical in one case and not identified in the remaining eight cases. Glandular involvement was found in nine patients.

Results of second cryosurgical treatment

The results of the second cryosurgical treatment are presented in Table 65.

Table 65. Results of the second cryosurgical treatment (N=38) and length of follow-up in patients with negative cytology in the Period II study

Time after treatment (in months)	A No. patients with failure	B No. patients with negative cytology	C No. patients not followed-up
Less than 6	6	32	0
6 - 11	0	32	0
12 - 23	1	30	1
24 - 35	0	25	6
36 - 47	0	12	19
48 - 59	0	8	23
60 - 71	0	1	30
72 - 83	0	0	31
Total no. of patients with failure	7		

Column A shows the number of patients with abnormal cytology in each period, Column B the number and length of follow-up in patients with negative smears and Column C the number of patients not yet followed-up in each period. Six of the seven patients classified as failures were detected at the first check-up less than six months after treatment. The mean observation time for the 31 patients without evidence of persistent disease was 3.2 years (range 0.8 - 5.1 years). The mean number of negative cytologic smears in each patient was 4.7. Further treatment was required in seven patients.

b. Cold-knife conization

After failure of the initial cryosurgical treatment indicated by abnormal cytologic findings, conization was performed in 16 patients. As mentioned above, a suspicious microinvasive carcinoma in the pretreatment biopsy specimen warranted conization in one additional patient. A repeat biopsy, ECC and, when indicated, an endometrial curettage was performed prior to conization in six patients for various reasons, e.g. cytologic smears indicating CIN 1 or to exclude progression to invasive carcinoma. A persistent CIN 3 lesion was found at the histopathological examination in these six patients. The conization technique was the same as that during the Period I study. Histopathological examination of the cone specimen showed no lesion in two patients, CIN 1 in one patient and CIN 2 or 3 in the remaining 14 patients. Among those patients with persistent disease (N=15), an exocervical lesion was found in eight patients, both exo- and endocervical lesion in four patients and an endocervical lesion in three patients, radically removed in all. Glandular involvement was found in seven patients.

Results of cold-knife conization

The results of the conization are shown in Table 66.

Table 66. Results of conization (N=17) after failure of the first cryosurgical treatment and length of follow-up in patients with negative cytology in the Period II study

	A	B	C
Time after conization (in months)	No. patients with failure	No. patients with negative cytology	No. patients not followed-up
Less than 6	1	16	0
6 - 11	0	16	0
12 - 23	0	14	2
24 - 35	0	11	5
36 - 47	0	7	9
48 - 59	0	6	10
60 - 71	0	2	14
72 - 83	0	2	14
84 - 95	0	0	16
Total no. of patients with failure	1		

Column A shows one patient with failure detected at the first check-up less than six months after conization. Column B shows the number and length of follow-up in patients with negative cytology in each period and Column C the number of patients not yet followed-up. The mean observation time after conization was 3.0 years (range 0.6 - 6.3 years). The mean number of negative smears after conization in each patient was 4.1. Further treatment was required in one patient.

c. Total hysterectomy

One patient, 25 years old, moved out of the region shortly after the cryosurgical treatment and was examined at an other hospital when abnormal smear was detected ten months after the initial cryosurgical treatment. Following a biopsy diagnosis of CIN 3, a total hysterectomy was performed 2.1 years after the initial treatment. Persistent CIN 3 was found with exo- and endocervical location in the hysterectomy specimen, radically removed. Five subsequent cytologic smears remained negative during a follow-up period of 2.4 years.

3. Management of persistent disease after two treatments

As mentioned above, seven patients were classified as failures after two cryosurgical treatments and one patient after one cryotreatment followed by conization. In the first seven failure cases mentioned, the first check-up smear after the second treatment was abnormal in six patients, whereas the remaining patient had two negative smears before the first abnormal one. All these patients were submitted to conization. In the cone specimen, no lesion was found in one patient, CIN 1 in one patient, CIN 2 in one patient and CIN 3 in the remaining four patients. The lesions were radically removed and followed by negative cytologic smears in all but one case with an endocervical lesion. Subsequent to the conization, this patient had an uneventful delivery followed by cytologic smear indicating CIN 3. After biopsy-confirmation of the diagnosis of CIN 3, a second conization was performed 2.1 years after the first one. A persistent CIN 3 lesion (exocervical location) was radically removed and followed by negative cytologic smears. The mean observation time after two cryosurgical treatments and one conization was 3.2 years (range 2.0 - 4.8 years). The mean number of negative smears after the conization in each patient was 5.0. The patient with two cryosurgical treatments and two conizations had three negative cytologic smears during an observation period of 1.1 year.

The eighth failure case (after one cryosurgical treatment followed by conization) had undergone a subtotal abdominal hysterectomy three years before cryosurgery. The cone biopsy was therefore performed as an amputation of the cervix. Subsequent to the cervical amputation, vaginal cytologic smears still indicated CIN 3, verified by colposcopically-directed biopsies from the vaginal wall. Resection of the vaginal wall was chosen as further treatment in this case. After the resection, six cytologic smears showed no evidence of persistent disease during a follow-up of 3.2 years.

4. Special histopathological follow-up study

As mentioned on p. 90, a special histopathological follow-up was performed for various reasons in selected cases. The procedure included extensive cervical biopsies, ECC and - when indicated due to abnormal bleeding - an endometrial curettage. Colposcopy was not regularly performed after the cryosurgical treatment. The intention was to perform this special check-up in patients who, despite negative or occasional smears indicating CIN 1, were considered to be a risk group. The main reasons for conducting this check-up were:

- unsatisfactory colposcopy prior to the initial treatment or colposcopic findings difficult to interpret after the treatment,
- endocervical location of the lesions or suspicious microinvasive carcinoma at the histopathological examination;
- patients above 35 years of age at the initial cryosurgery and patients submitted to a second cryosurgical treatment;
- abnormal bleeding;
- occasional cytologic smear indicating CIN 1 after treatment;
- patients anxious about persistent disease.

This special histopathological follow-up was performed a total of 64 times in 56 patients. The procedure was done within one year after cryosurgery on 18 occasions, between one and two years on 27 occasions and between three and five years on the remaining 19 occasions. No evidence of persistent disease was found at histopathological examination in 47 patients. A CIN 1 was found in eight patients (six of them had a previous smear indicating CIN 1 and two of them had previous negative smears). A CIN 2 lesion was found in the remaining patient. That patient had undergone two cryosurgical treatments and one conization. Subsequent cytologic smears were negative, and no further treatment was thought to be required.

Besides the 56 patients, three additional patients offered the possibility for complete examination of the cervix after cryotreatment, as a hysterectomy was performed in these three cases because of other gynaecologic disorders. One of these had a persistent CIN 1 lesion (see p. 108), whereas the other two lacked evidence of persistent cervical disorder.

5. Reproduction after cryosurgery

In the Period II study, 89 women had 113 pregnancies begun subsequent to cryosurgical treatment, and one additional woman was pregnant at the time of the first cryosurgical treatment. The outcomes of the 114 terminated pregnancies are shown in Table 67.

Table 67. Pregnancy outcome following cryosurgery in the Period II study

<u>Pregnancy outcome</u>	<u>No. pregnancies</u>
Full-term pregnancy	79
Pre-term delivery	4
Spontaneous abortion (at <16 weeks)	10
Legal abortion (at <12 weeks)	20
Ectopic pregnancy	1
Total no. of pregnancies	114

Seventy-two women had a total of 83 deliveries: Sixty-three had one delivery (4 had twin-deliveries), seven had two deliveries and two had three deliveries. Five women became pregnant after two cryosurgical treatments, four after one cryosurgery and conization and the remaining 63 women after one cryosurgical treatment. The time interval between the first cryosurgical treatment and conception, as calculated from the last menstrual period, is shown in Table 68.

The mode of delivery included 69 normal vaginal deliveries, 11 cesarean sections and three vacuum extractions. Cervical dystocia was not present in any of these deliveries. There was no perinatal mortality in the 83 delivery cases. Notably, four women had a twin pregnancy.

Table 68. Interval between first cryosurgical treatment and conception as calculated from LMP in the Period II study

Time: cryosurgery-conception	
(in months)	No. pregnancies
Less than 6	17
6 - 11	20
12 - 23	29
24 - 35	28
36 - 47	15
48 - 59	4
Total no. of pregnancies	113

SUMMARY AND COMBINED EVALUATION OF THE PERIOD I STUDY AND THE PERIOD II STUDY.
DISCUSSION OF RESULTS.

A. General remarks on the material

The present investigation is a thorough study of the clinical value of cryosurgical treatment of cervical intraepithelial neoplasia (CIN). The investigation, carried out at the Department of Obstetrics and Gynaecology, Central Hospital, Kristianstad, is based on two different three-year series of patients, these series subsequently referred to as the Period I study and the Period II study, respectively. The majority of the patients were evaluated by colposcopic assessment, directed biopsy and ECC. The cryosurgical treatment was performed regardless of the extension of the transformation zone and the colposcopic visibility of the lesions.

In the Period I study (material collected 1973-1975), the effects of cryosurgical treatment were studied in patients fulfilling the following criteria: below 40 years of age, histopathologically verified CIN 3 and persistent or recurrent post-biopsy abnormal cytologic findings. Complications and side-effects related to cryosurgery were also studied. The cytologic and histopathological slides were reviewed by the same pathologist. The majority of the diagnostic procedures and follow-ups and all cryosurgical treatments were performed by the author. The series is characterized by an extremely low drop-out rate, this due to the effective population register system in Sweden. All residents can be traced, and information about the results of cytologic smears taken at other places in the country can almost always be obtained from the departments of gynaecology or cytologic laboratories.

The Period II study (material collected 1976-1978) represents a practical, clinical application of cryosurgery as a routine treatment of patients fulfilling the following criteria: reproductive age, cytologic smears indicating CIN 1 to 3, and histopathologically confirmed CIN 3. Even though patients with CIN of all degrees were treated, only those patients with histopathologically verified CIN 3 are presented. The clinical evaluations, treatments and follow-ups in this material were done to a major extent by the author.

In the Period I study, 201 patients with a biopsy-confirmed CIN 3 were candidates for cryosurgery. However, 30 patients had persistently negative post-biopsy cytologic smears; thus 171 patients fulfilled the criteria mentioned above for being submitted to cryosurgical treatment.

In the Period II study, 332 patients with a histopathological diagnosis of CIN 3 fulfilled the criteria established for being included in the study. They were managed according to three different alternatives:

Alternative I (N=249). Cryosurgery performed in association with the diagnostic procedures.

Alternative II (N=32). Cryosurgery after the diagnostic report was obtained.

Alternative III (N=51). Cryosurgery restricted to cases with abnormal post-biopsy cytologic findings.

One hundred and twenty-one patients with a histopathological diagnosis of CIN 3, who did not fulfil the criteria for being included in any of the study groups mentioned, were treated differently: 89 (73.6 %) had conization and 18 (14.9 %) had total hysterectomy. The remaining patients were treated with various other methods.

B. Detection of patients and histopathological diagnosis

In the total series of 533 patients with a histopathological diagnosis of CIN 3, 529 (99.2 %) were detected by abnormal cytologic smears. During the Period I study, when a screening program existed in the area, 28 per cent of the patients were detected by screening. These findings may be compared to a report from Uppsala, according to which general screening contributed 23 per cent of the patients with carcinoma in situ (72). In the Period II study, when the general screening was running only during the first year of the Period, 16 per cent were detected by screening.

For a considerable number of patients, repeated negative cytologic smears were obtained before the detection of abnormal smear. The initial smear was negative in 47.7 % of the patients: 25.0 % had one, 12.5 % had two, 6.8 % had three and 3.4 % had four or more negative smears before the first abnormal smear. The gynaecological health check-ups took place at four-year intervals but the average time between repeated negative cytologic smears was about three years because a new cytologic smear was taken at examinations in association with pregnancy, contraceptive advice or gynaecological illness, if more than one year had elapsed since the previous smear was taken.

Before the Period I study started, the policy of the Department was that all patients with cytologic smears indicating CIN 3 were hospitalized and treated by conization. Because of both immediate and later complications of conization, this treatment was not performed until repeated smears indicating CIN 3 were obtained. This explains why so many of the patients in the Period I study had repeated cytologic smears indicating CIN 3 before the patients were included in the study.

During the course of the Period I study, more and more patients, according to the design, were included in the study right after the first cytologic smear indicating CIN 3 without a repeat smear. In addition, patients with repeated cytologic smears indicating CIN 1 and 2 were included, as it was observed during the course of the study that even these patients could have CIN 3 at the histopathological examination (see Table 21, p. 52 and Table 54, p. 100). The time from the first abnormal cytologic smear until the histopathological diagnosis of CIN 3 was 25 months on the average if the first cytologic smear showed CIN 1 or 2, and 16 months if it showed CIN 3. The most advanced cytologic smear prior to the histopathological diagnosis of CIN 3 indicated CIN 1 or 2 in 28 per cent of the cases and CIN 3 in 72 per cent.

During the Period II study, the design was changed so that even patients with cytologic smears indicating CIN 1 and 2 were immediately examined histopathologically. However, since cytologic smears suggesting CIN 1 can be difficult to differentiate from inflammatory or metaplastic changes, and spontaneous remission occurs at a high rate, the CIN 1 smear was in general repeated after three months, or possibly after treatment for colpitis. The histopathological examination was done only if cytologic changes remained.

At the end of the Period II study, when the design was completely implemented, the time from the first abnormal smear indicating CIN 1 (21 %), CIN 2 (38 %) and CIN 3 (41 %) to histopathological diagnosis of CIN 3 was 8, 6 and 4 months, respectively. If a one-degree difference between cytologic and histopathological findings is accepted, these findings indicate that cytologic smears were underestimated in 21 per cent of the patients, which is in accordance with earlier reports (108, 150).

All patients with abnormal cytology were referred to extensive diagnostic evaluation according to the design, which differed in some details between the Period I study and the Period II study (p. 37 and p. 89). The diagnostic procedures included biopsy and, in most cases, ECC and colposcopy. The colposcopic examination was performed in order to exclude invasive carcinoma, to take biopsy from the most abnormal transformation zone and to assess the location of the lesions. Among the 533 patients included in the Period I and II studies, a histopathological diagnosis of CIN 3 was obtained in 528 patients (99.1 %), a suspected though not confirmed microinvasive carcinoma in four patients (0.7 %) and an adenocarcinoma of the uterine cervix in one patient (0.2 %). When invasive carcinoma was colposcopically excluded, the patients were referred to further management according to the designs of the Period I study and the Period II study, respectively.

C. Cryosurgical treatment, side-effects and complications

The initial cryosurgical treatment was performed with a standardized double-freeze technique. Nitrous oxide was used as refrigerant. All patients were treated on an out-patient basis. Any intrauterine device was left in place during treatment. Local infiltration anaesthesia, used in 40 per cent of the patients in the Period I study, was replaced by local spray anaesthesia in the Period II study, and was subsequently used in all patients. A few patients had lower abdominal pain during treatment but the treatment could be completed in all cases.

Following treatment, 49 per cent of the patients experienced moderate vasomotor reactions. All patients reported vaginal discharge during the first week, this gradually subsiding during the next few weeks. Bloody discharge appeared in 25 per cent of the patients and most frequently in those treated in the premenstrual period. Malodorous discharge was reported in 30 per cent and contact-bleeding in five per cent after treatment. A few patients had menstrual-like lumbar pain the first two days following treatment. General weakness was experienced in about 20 per cent of the patients, usually during the first week after treatment. Sick-leave was restricted to patients who could not change pads during working-hours. A temporary increase in body temperature was reported by some patients, though no signs of pelvic inflammatory disease were found in these patients. No patient with a previous history of PID developed acute salpingitis after cryosurgery. Two patients had laparoscopy-verified PID more than six months after the cryosurgical treatment.

No case of cervical stenosis occurred after cryosurgery, though some reduction of the diameter of the cervical canal was detectable in four per cent of the patients at examinations between one and seven years after treatment. No other side-effects or complications with any possible relation to cryosurgery were found during the entire follow-up period.

There was no evidence that cryosurgery had any adverse effects on fertility or on the outcomes of pregnancies occurring up to the present time. A total of 150 women had 194 (mean 1.3) post-cryosurgical conceptions. The interval between cryosurgery and conception varied from a few weeks to approximately eight years after treatment. The average duration of both labour and its second stage did not show any specific deviation from the total obstetrical material of the Department. The frequency of cesarean sections and minor obstetric surgery was even somewhat lower than the average at the Department in the corresponding period of time. No significant influence was found on the frequency of pre-term deliveries and spontaneous abortions.

D. Follow-up examination

Subsequent to treatment, the patients were followed-up at 3, 6, 12 months, and then once a year, providing no signs of persistent or recurrent disease were detected. In addition, a special histopathological follow-up study was performed routinely in the Period I study and, according to the design, in selected cases in the Period II study. Ninety-two (96 %) of the 96 failure cases were detected by abnormal cytology, and the remaining four cases (4 %) at this histopathological follow-up study despite repeated negative cytologic findings. As colposcopic examination was unsatisfactory in a considerable number of patients (24 % after one and 71 % after two cryosurgical treatments), colposcopy was gradually excluded from the follow-up protocol.

The concept of failure rate

One of the most difficult problems in evaluating the results of cryosurgical treatment of CIN concerns definition of failure and failure rate. This concerns the results of a treatment of an illness with a long natural history; progression from CIN 1 to CIN 3 has been calculated to take about seven years, and from CIN 3 to invasive carcinoma 10 years or more (14, 117, 134). The results thus cannot be expressed in terms of cure rate unless the follow-up

period extends over a very long time. In most reports the results are expressed in total failure rate, which generally means that the number of patients with failure is calculated per the number of patients treated, but without consideration of the patients lost for follow-up or the length of the follow-up period.

The design of the present investigation permits the analysis of failure rate in a detailed manner. As the patient material had to be collected during a long time, the results must be evaluated in patient groups with the same follow-up time after treatment. Since not all patients could be followed the same amount of time and some patients were even lost to follow-up, there is an increased difference between the number of patients followed-up and those not yet followed-up, this being especially so in those groups with a long follow-up time. It is reasonable to assume that, if less than 5 % are not yet followed-up in each period, the results can be considered as reliable; at a frequency of 5 - 20 % not yet followed-up in a period, one can get an impression of the results; however, if more than 20 % are not followed-up, the evaluation of the results becomes too uncertain.

In order to be able to judge the effect of cryosurgical treatment of CIN per se, the study's design must be such that confirmation is obtained that the lesions remain after the diagnostic procedures and prior to treatment. Only a few studies which fulfil these requirements have been published previously (40, 43, 64, 154, 163, 167). In the current investigation the Period I study met the conditions necessary for judging the cryosurgical effect in treatment of lesions which remain after biopsy, as only patients with post-biopsy abnormal cytologic findings were treated. Even patients in the Period II study who were managed according to Alternative III fulfilled these requirements of remaining abnormal cytologic smears after the biopsy; however, these patients constitute a selected group (small lesion at colposcopic examination). The results of treatment of patients in the Period II study who were managed according to Alternative I and II represent, on the other hand, the combined effects of biopsy, ECC and cryotreatment.

In the report of the results, the definitions of failure and failure rate given on p. 37 for the Period I study were applied in the Period II study too.

In calculating the results, the following definitions and formulas were used in each follow-up period:

A = number of patients with failure in that period

B = number of patients with negative cytology or histopathology in that period

C = number of patients, who were not yet followed-up or who were lost for follow-up in that period.

$$\text{Real Failure Rate (RFR)} = \frac{A \times 100}{A + B + C} \quad (\text{where C per definition is } = 0)$$

$$\text{Preliminary Failure Rate (PFR)} = \frac{A \times 100}{A + B}$$

$$\text{No-Follow-up Rate (NFR)} = \frac{C \times 100}{A + B + C}$$

Corrected Failure Rate (CFR) = failure rate after more than one cryosurgical treatment

In addition, the results after the diagnostic biopsy and after cryosurgical treatment were analysed and reported according to traditional methods of life table calculations. Calculations of the results per the above yields a reliable, comparable and honest expression of the method's effectiveness relevant to varying follow-up times and the number of patients followed-up.

E. Results of the Period I study and the Period II study

1. Results of the first cryosurgical treatment

Before the results of the cryosurgical treatment are presented, the therapeutic effects of the biopsy per se are given for patients with a repeat post-biopsy cytologic smear in the Period I study. Patients with abnormal cytologic findings after the biopsy are recorded as failures, and the failure rate is calculated in a manner similar to that after cryosurgery, as shown in Table 69.

Table 69. Frequency of persistent or recurrent disease after diagnostic biopsy in the Period I study (Compiled from Table 24, p. 56)

Time after biopsy (in months)	RFR	PFR	NFR
Less than 6	72.1	-	0
6 - 11	23.2	-	0
12 - 23	14.0	-	0
24 - 35	16.2	-	0
36 - 47	0	-	0
48 - 59	-	3.2	3.2
60 - 71	-	0	6.7
72 - 83	-	0	13.3
84 - 95	-	0	> 20

At examination within six months after the biopsy, abnormal smear indicating persistent disease was found in 72.1 per cent of the patients. During the follow-up the cytologic smear reverted to abnormality in a number of cases. However, after five years' follow-up, no more cases with abnormal cytology were detected up to the time the investigation was finished. During a mean follow-up of 7.0 years after the biopsy, 14.9 per cent of the patients with a histopathologically confirmed diagnosis of CIN 3 still had negative cytologic smears and were apparently effectively treated by the biopsy alone.

Patients with abnormal post-biopsy cytologic findings (171 in the Period I study and 51 managed according to Alternative III in the Period II study) were referred to cryosurgical treatment. The results of the cryosurgical treatment of these patients thus reflected the effects of cryosurgery per se, as it was the sole method of treatment.

Table 70 presents the results of the first cryosurgical treatment. In the Period I study, a RFR of 15.2 per cent was found at examination within six months after treatment, and in the Period II study (Alternative III) a RFR of 5.9 per cent was found. The difference in failure rate between these two groups of patients appears to be large but is not statistically significant ($p=0.08$). Those patients in the Period II study who were managed according to Alternative III consisted of a selected group with small, colposcopically visible lesion.

Table 70. Results of first cryosurgical treatment in patients with abnormal post-biopsy cytologic findings (N=222) (Compiled from Table 34, p. 72 and Table 61, p. 107).

Time after treatment (in months)	Period I study (N=171)			Period II study Alternative III (N=51)		
	RFR	PFR	NFR	RFR	PFR	NFR
Less than 6	15.2	-	0	5.9	-	0
6 - 11	3.4	-	0	-	0	2.1
12 - 23	4.3	-	0	-	0	6.3
24 - 35	-	1.5	0.7	-	0	8.3
36 - 47	-	0.8	1.5	-	0	>20
48 - 59	-	0	6.1			
60 - 71	-	0	>20			

No signs of disease were found in 76.6 per cent of the patients in the Period I study during a mean follow-up of 6.1 years or 94.1 per cent of the patients in the Period II study managed according to Alternative III during a mean follow-up of 3.0 years.

The results of the combined effects of biopsy and the first cryosurgical treatment of patients in the Period II study managed according to Alternative I and II (biopsy-confirmed CIN 3 without repeat smear) are presented in Table 71.

Table 71. Results of the first cryosurgical treatment in patients with biopsy-confirmed CIN 3 without post-biopsy cytologic smear (Compiled from Table 59, p. 105 and Table 60, p. 106)

Time after treatment (in months)	Period II study					
	Alternative I (N=249)			Alternative II (N=32)		
	RFR	PFR	NFR	RFR	PFR	NFR
Less than 6	-	12.1	0.4	12.4	-	0
6 - 11	-	2.8	0.9	0	-	0
12 - 23	-	2.4	1.4	1.6	-	0
24 - 35	-	2.0	5.3	0	-	0
36 - 47	-	1.2	18.1	-	0	3.7
48 - 59	-	1.0	> 20	-	0	> 20

There was no statistically significant difference in failure rate between the two groups of patients managed according to Alternative I (cryosurgery in association with biopsy) and Alternative II (cryosurgery some time after biopsy), respectively. The somewhat lower failure rate in the Period II study (Alternative I and II) compared with the Period I study corresponds approximately to the therapeutic effects of the biopsy.

No signs of disease were found in 80.7 per cent of the patients managed according to Alternative I during a mean follow-up of 3.8 years and in 84.4 per cent of the patients managed according to Alternative II during a mean follow-up of 4.4 years. The life-table calculations of the results of the various managements of the patients are shown in Fig. 16.

After the first cryosurgical treatment, all 96 patients classified as failures (40 in the Period I study and 56 in the Period II study) were assigned to further treatment, as shown in Table 72.

Fig. 16. Life table analysis of patients with failure after the diagnostic biopsy and after the first cryosurgical treatment in the Period I study and the Period II study (Alternatives I, II and III).

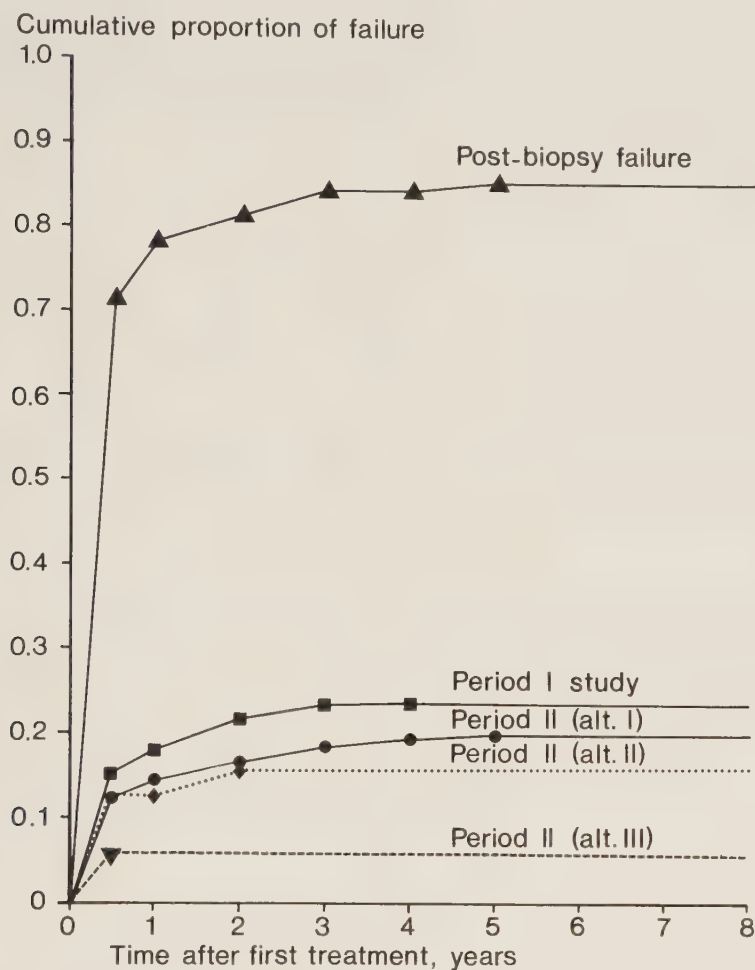


Table 72. Further treatment of patients with failure after the first cryosurgical treatment

Method of treatment	No. patients
Second cryosurgical treatment	60
Conization	31
Biopsy followed by negative cytology	3
Total hysterectomy	2
Total	96

2. Results of the second cryosurgical treatment

After failure of the first cryosurgical treatment, 60 patients (22 in the Period I study and 38 in the Period II study) were submitted to a second cryosurgical treatment performed in the same way as in the first one, with the exception that seven patients received an additional endocervical freezing. In about half of the patients, the persistent disease was confirmed by abnormal cytologic smear prior to the second cryosurgery. The results of the second cryosurgical treatment are shown in Table 73.

Table 73. Results of the second cryosurgical treatment (N=60)

Time after treatment (in months)	RFR	PFR	NFR
Less than 6	16.7	-	0
6 - 11	0	-	0
12 - 23	-	2.0	2.0
24 - 35	-	0	12.2
36 - 47	-	0	> 20

A RFR of 16.7 was found at examination within six months after treatment. There was no statistically significant difference between the results of the first cryosurgical treatment versus the results of the second cryosurgical treatment.

No signs of disease were found in 81.7 per cent of the patients after the second cryosurgical treatment during a mean follow-up of 3.9 years. Patients with failure were referred to conization.

3. Corrected failure rate

Corrected failure rate (CFR) is defined as the percentage of patients with failure after more than one cryosurgical treatment (31). In the present study no more than two cryosurgical treatment sessions were performed in any individual patient. The CFR in the Period I study, the Period II study and in the total material is presented in Table 74.

Table 74. Corrected failure rate in the Period I study, the Period II study and the total material

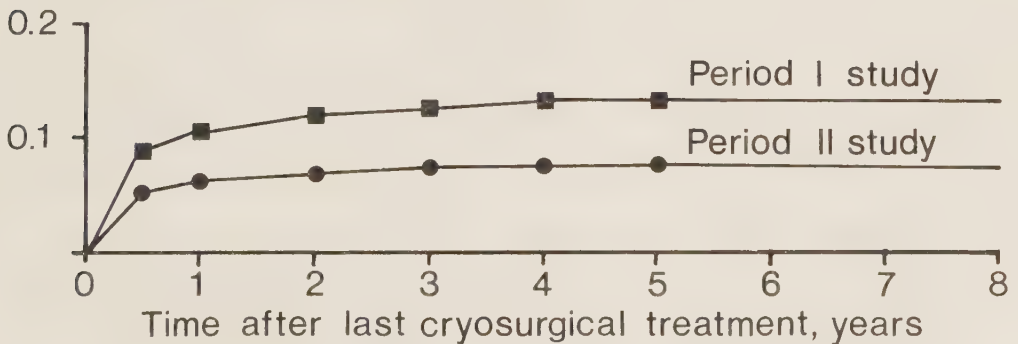
Failure period after the first cryosurgical treatment (in months)	Period I study (N=171)			Period II study (N=332)		Total material (N=503)	
	RFR	PFR	NFR	PFR	NFR	PFR	NFR
Less than 6	8.8	-	0	5.1	0.3	6.4	0.2
6 - 11	1.9	-	0	1.0	1.0	1.3	0.6
12 - 23	1.3	-	0	0.7	2.3	0.9	1.5
24 - 35	-	0.7	0.7	0.3	6.5	0.5	4.6
36 - 47	-	0.7	1.3	0.4	>20	0.5	17.2
48 - 59	-	0	6.7			0.7	>20
60 - 71	-	0	16.8				
72 - 83	-	0	>20				

No signs of disease after cryosurgery were found in 90.7 per cent of the patients during a mean follow-up of 4.4 years after the last cryosurgical treatment.

Fig. 17. shows the life table analysis for patients with failure after cryosurgery (CFR) in the Period I study and the Period II study, respectively.

Fig. 17. Life table analysis of patients with failure after the last cryosurgical treatment in the Period I study and the Period II study

Cumulative proportion of failure



4. Results of conization, biopsy and total hysterectomy

After failure of the first cryosurgical treatment, conization was performed in 31 patients (14 in the Period I study and 17 in the Period II study). The results of the conization are shown in Table 75.

Table 75. Results of conization (N=31) after failure of the first cryosurgical treatment

Time after conization (in months)	RFR	PFR	NFR
Less than 6	9.7	-	0
6 - 11	-	3.8	7.1
12 - 23	-	0	14.8
24 - 35	-	0	> 20

A RFR of 9.7 per cent was found at examination within six months after the conization. No signs of disease after conization were found in 87.1 per cent of the patients during a mean follow-up of 3.8 years.

After failure of the first cryosurgical treatment, a diagnostic biopsy was followed by negative cytologic smears in three patients.

A total hysterectomy with subsequent negative cytologic findings was the final treatment in two patients with failure after the first cryosurgical treatment.

5. Further treatment of persistent disease

Persistent disease after two cryosurgical treatments was found in 11 patients, and after one cryosurgical treatment and one conization in four patients. All 15 patients were treated individually and were without signs of disease when the investigation was concluded.

6. Survey of various types of treatment

A comprehensive survey of the lengths of follow-up after the final treatment and the various types of treatment and combination of treatment methods required for removal of the neoplastic changes is presented in Table 76.

Table 76. Survey of final treatment and length of follow-up of the total material (N=533)

Final treatment	Period I	Period II	Total No.	Mean follow-up (in years)
	study No.	study No.		
Biopsy followed by negative cytology	30		30	7.0
One cryosurgical treatment	131	276	407	4.5
One cryosurgical treatment and biopsy	3		3	4.7
One cryosurgical treatment and one conization	11	16	27	3.8
One cryosurgical treatment and two conizations	1		1	4.5
One cryosurgical treatment and hysterectomy	1	1	2	3.2
One cryosurgical treatment, one conization and hysterectomy	1		1	0.8
One cryosurgical treatment, two conizations and hysterectomy	1		1	1.6
One cryosurgical treatment, cervical amputation and vaginal resection		1	1	3.2
Two cryosurgical treatments	18	31	49	3.9
Two cryosurgical treatments and one conization	3	6	9	3.7
Two cryosurgical treatments and two conizations	1	1	2	2.6
Total no. of patients	201	332	533	

Of the total number of 533 patients included in the present investigation, the final treatment given was cryosurgery in 456 patients (85.5 %), conization in 39 patients (7.3 %), biopsy in 33 patients (6.2 %), total hysterectomy in four patients (0.8 %) and vaginal resection in the remaining patient (0.2 %).

CONCLUSIONS AND GENERAL REMARKS

The results of the present investigation strongly suggest that the cryosurgical treatment of patients with a histopathological diagnosis of CIN 3 is highly effective. The present investigation stands in contrast with most other published reports in the literature, in that it contained a uniform and thorough pretreatment evaluation, histopathologically confirmed diagnosis of CIN 3, standardized freezing-technique, clearly defined criteria for failure and failure rate, and long-term cytologic and histopathological follow-up data. The investigation has shown that most of the failure cases can be treated effectively by a second cryosurgical session.

The experience obtained in this long-term follow-up study has allowed the establishment of guide-lines for the management of patients with CIN and for the application of cryosurgery in clinical practice, including further treatment of the few cases with failure after cryosurgery. Abnormal cytologic smear surely only suggests some degree of cervical neoplasia but does not necessarily indicate the precise degree of abnormality of the lesion, as compared with subsequent histopathological examination. Exclusion of invasive cervical carcinoma by means of a thorough diagnostic evaluation is an absolute requirement prior to performing cryosurgery. An immediate clinical evaluation of patients with abnormal cytologic smear without repeated check-ups can save these patients much concern and anxiety, and save the gynaecological out-patient departments and the cytologic laboratories much unnecessary work.

The diagnostic procedures including colposcopic examination, directed biopsy and ECC are highly effective in evaluation of the nature and extent of the lesions. In some patients the diagnostic procedures per se may be effective in removing the lesions, particularly in low-parity women without glandular involvement. When invasive carcinoma is colposcopically ruled out, cryosurgery has an optimal therapeutic effect, with a minimum of adverse somatic and psychologic side-effects. Cryosurgery is generally considered as contraindicated in patients with unsatisfactory colposcopy. However, when thorough diagnostic evaluation, including ECC, has been performed and when cryosurgery is restricted to patients below 40 years of age, the very few patients with unsatisfactory colposcopy have the same low failure rate as those with satisfactory colposcopy.

The results clearly suggest that cryosurgery is the method of choice in patients below 30 years of age. In patients between 30 and 40 years of age, the choice of treatment method will depend upon the colposcopic findings, the desire for preserving childbearing capacity and the patient's attitude toward a conservative method of treatment. Nevertheless, the majority of patients in this age range can be effectively and safely treated with cryosurgery. Patients 40 years or older should be referred to diagnostic conization or, in selected patients, cryosurgery.

The great advantage of cryosurgical treatment is that it is applied on an out-patient basis without general anaesthesia. The procedure is as a rule painless; it is associated with minimal bleeding; it is effective and safe for the patients; and complications are negligible. No impairment of fertility and reproductive outcome has been found. The equipment is easy to handle, reliable, and inexpensive to purchase and operate. In case of failure of the first cryosurgical treatment, a second session can be performed after a repeat diagnostic evaluation. Conization or total hysterectomy may be necessary in only a few patients.

The follow-up after cryosurgery is extremely important. Traditionally, patients are followed-up at three-month intervals after cryosurgery. However, as cytologic smear findings within three months after treatment may be confused with dysplasia due to repair processes in the epithelium, the first smear should be taken four months after treatment, and the next smear after another four months. According to experiences from this study, colposcopy frequently is unsatisfactory after cryosurgery and as cytologic smears may be false negative, a histopathological examination including biopsy and ECC is recommended for inclusion in the follow-up examination about one year after treatment. When these check-ups yield no signs of persistent disease, the patients are followed-up with cytologic smear once a year thereafter.

The present investigation did not include a systematic comparison of cryosurgical treatment with other conservative methods. However, when cryosurgery is performed according to the present management conditions, it is no doubt a highly useful addition to traditional methods for treating CIN.

No definite reason for failure after cryosurgery has been identified. It might be that conditions which predispose the development of CIN are still present after cryosurgery and will continue to exert influence on the vulnerable epithelium of the uterine cervix. As the progression from CIN to invasive carcinoma takes a long time, it is not yet known whether cryosurgery simply postpones the progression of the epithelial changes. It has been suggested that pathological epithelium not completely destroyed by cryosurgery may remain buried in the stoma, if the surface is covered by normal squamous epithelium (22). In such case, follow-up cytology will be negative and after many years epithelium may progress to invasive carcinoma. However, no evidence of such hidden epithelium has so far been found in the cases undergoing conization or hysterectomy in the present investigation. The risk of missing invasive carcinoma seems to be minimal in properly selected patients, who receive accurate diagnostic evaluation and follow-up examination including cytology and histopathology. In cases of failure of cryosurgery, a more extensive diagnostic or therapeutic procedure such as cone biopsy or total hysterectomy can always be performed.

GENERAL SUMMARY

The present investigation studied the long-term effects of cryosurgical treatment of patients with a histopathological diagnosis of CIN 3 (severe dysplasia and carcinoma in situ of the uterine cervix). In addition to cytology the diagnostic procedures in a majority of the patients included colposcopic examination, directed biopsy and endocervical curettage. A double-freeze technique was employed with two three-minute periods of freezing. Nitrous oxide was used as refrigerant. All patients were treated on an out-patient basis. The material was collected at the Department of Obstetrics and Gynaecology, Central Hospital, Kristianstad, during two three-year periods 1973-1978. In the Period I study (material collected 1973-1975), 201 consecutive patients below 40 years of age with a histopathologically confirmed CIN 3 were candidates for cryosurgery. Following diagnostic biopsy, 30 patients (14.9 %) had negative cytologic findings during a mean length of follow-up of 7.0 years and were not given any further treatment; 171 patients (85.1 %) had abnormal post-biopsy cytologic findings and were referred to cryosurgical treatment.

After the first cryosurgical treatment, 131 patients (76.6 %) were without signs of disease during a mean of 6.1 years' follow-up; 40 patients (23.4 %) were classified as failures and treated with different methods: 22 patients were treated with a second cryosurgery, 14 patients were treated with conization and one patient with total hysterectomy. The remaining three patients had negative cytologic findings after a repeat diagnostic biopsy. The further management of the patients classified as failures after two treatments is illustrated in the flow chart for the Period I study, presented in Fig. 18.

HIS

POST-BIOPSY NEG
(30)

TREATMENT:

NEG.
(131)

TREATMENT:

SECOND CRYOSUR
(22)

NEG.
(18)

ABN.
(4)

TREATMENT:

CONIZATIO
(4)

NEG.
(3)

ABN.
(1)

TREATMENT:

SECOND CONIZA
(1)

NEG. = negative (no further treatment require

ABN. = abnormal (persistent or recurrent dise

TOPATHOLOGICALLY CONFIRMED CIN 3
(201)

ATIVE

POST-BIOPSY ABNORMAL
(171)

CRYOSURGERY
(171)

ABN.
(40)

GERY

CONIZATION
(14)

BIOPSY
(3)

TOTAL HYSTERECTOMY
(1)

NEG.
(11)

ABN.
(3)

NEG.
(4)

N

SECOND CONIZATION
(2)

TOTAL HYSTERECTOMY
(1)

NEG.
(1)

ABN.
(1)

NEG.
(1)

ATION

TOTAL HYSTERECTOMY
(1)

NEG.
(2)

d)

ase)

Fig. 18. Flow chart for the Period I study (1973-1975). Figures indicate the number of patients.

In the Period II study (material collected 1976-1978), 332 consecutive patients of reproductive age with a histopathologically confirmed diagnosis of CIN 3 were given cryosurgical treatment and managed according to three different alternatives:

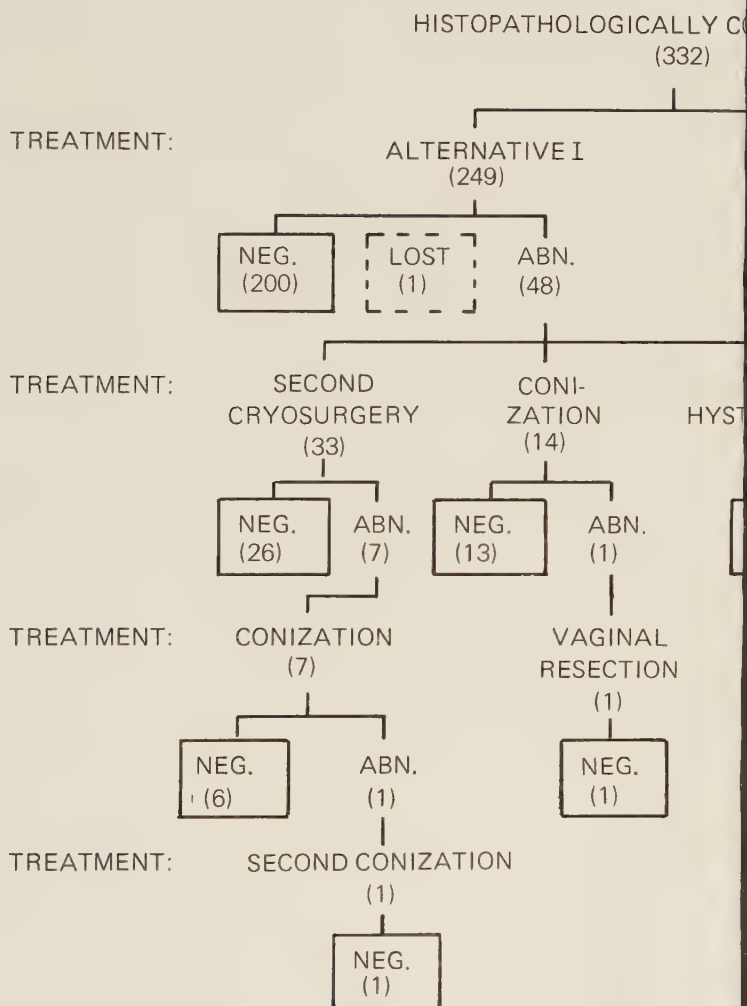
Alternative I (cryosurgery in association with biopsy and ECC) included 249 patients (75.0 %);

Alternative II (cryosurgery after the diagnostic report was obtained) included 32 patients (9.6 %);

Alternative III (cryosurgery of patients with abnormal post-biopsy cytologic findings) included 51 patients (15.4 %).

The failure rate after the first cryosurgical treatment was 19.3 %, 15.6 % and 5.9 % when managed according to Alternative I, II and III, respectively. The mean length of follow-up of patients without signs of disease after the first cryosurgical treatment was 3.8 years (Alternative I), 4.4 years (Alternative II) and 3.0 years (Alternative III). In the Period II study, 56 patients (16.9 %) were classified as failures after the first cryosurgical treatment and were given further therapy: 38 patients were treated with a second cryosurgery, 17 patients were treated with conization and one with total hysterectomy. The further management of patients with failure after two treatments is illustrated in the flow chart for the Period II study, presented in Fig. 19.

Thus, during the entire study period, 503 patients with a histopathologically confirmed diagnosis of CIN 3 were treated with cryosurgery; 96 (19.1 %) were classified as failures after the first cryosurgical treatment. Sixty of the failure cases underwent a second cryosurgical treatment, 11 (18.3 %) being classified as failures after the second cryosurgery. The corrected failure rate after cryosurgery was 9.3 % in the total series. No serious side-effects or complications following cryosurgery were observed. No evidence of reduced fertility or negative effects on the outcome of pregnancies or on the duration of labour was found. The conclusion from this long-term study is that cryosurgery is a safe, effective and inexpensive method for treatment of CIN 3 in patients below 40 years of age. However, it is of utmost importance that a precise diagnosis be established prior to treatment and that a meticulous follow-up be performed after treatment.



NEG. = negative (no further treatment required)
ABN. = abnormal (persistent or recurrent disease)

CONFIRMED CIN 3

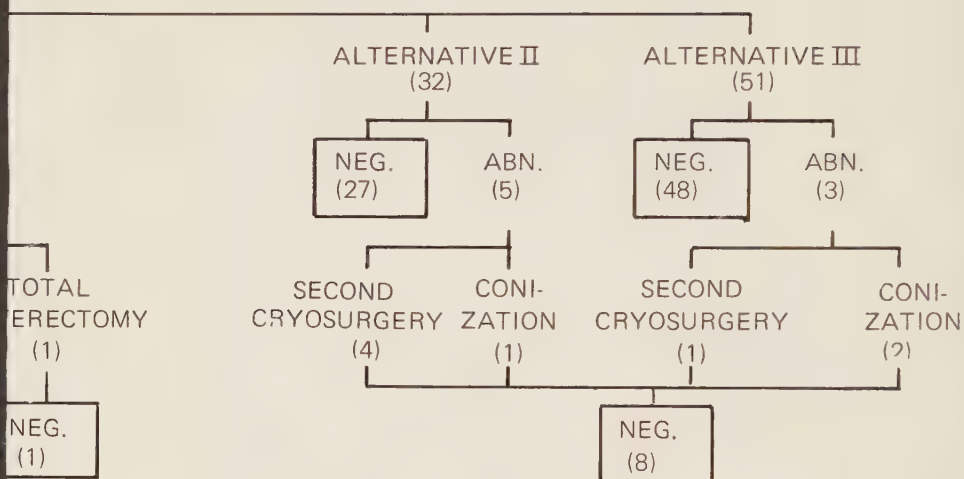


Fig. 19. Flow chart for the Period II study (1976-1978). Figures indicate the number of patients.

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